REMARKS

AMENDMENTS

IN THE CLAIMS

Applicants present amendments to claims 12 and 22 which effect changes requested by the examiner. In addition, applicants introduce new claims 24-27, and respectfully request that they be entered. These claims are limited to the present process wherein the new catalytic activity is within that IUB class already assigned to the original enzyme. Support for this new limitation is found in the specification at page 4, line 10, which introduces the IUB classification system, and from the example beginning on p.11, in which the new catalytic activity is within the IUB class assigned to the enzyme originally. It is respectfully urged that the claims are in *prima facie* condition for allowance, as the subject matter is novel and unobvious (the claims are based on extant claims which are no longer rejected over prior art), and is supported and enabled by the specification as originally filed. Entry will pose no additional burden on the examiner.

IN THE SPECIFICATION

Applicants again amend Table I on page 10 of the specification. This amendment is to introduce and/or clarify the proper trade names for the products referred to therein upon filing. Support for the amendment comes from the inherency of these names, as further set forward below. Additionally, portions of the specification

previously amended have been amended again to more clearly indicate applicants' intent.

OBJECTIONS TO THE SPECIFICATION

PARENTHETICAL STRUCTURE

In amending the parenthetical structure objected to, applicants intended that the equals sign, "=," be removed. Consistent with current amendment requirements, this sign has been struck through, like so "≡." Accordingly, it should not have been retained. In each instance of the noted parenthetical structure, applicants have attempted to replace the "=" with "i.e.," or some similar term, or have simply let the parentheses serve their common grammatical function(s). It is respectfully submitted that the present amendments clarify this situation.

REDUCTION OF K_M/INCREASE OF K_{CAT}

It is respectfully submitted that a reference value for K_m and/or k_{cat} is unnecessary for present purposes. In the present invention, the relative change is to be observed qualitatively, and the numerical value of the change is not important. As long as one of skill in the art can recognize that a catalytic activity was not present, using available detection methods, and subsequently recognize that the same catalytic activity *is* present, the new catalytic activity has been generated. Whether this is due specifically to a reduction in K_m or an increase in k_{cat}, and to what precise extent it is due

to either, is not necessary for one of skill in the art to know.

TRADE NAMES OF ENZYMES

The amendments to Table I on page 10 of the specification introduced the trade names of the commercially available enzymes employed in the examples beginning on page 9. Attached hereto are printed pages from various sources which indicate that the terms Amano, Lipoxyme, and Novozyme (amended herein to "Novozym," which is technically more correct) would be inherently observed by one of skill in the art when reading the information in the table as originally filed.

Applicants attach pages from the Amano Enzyme, Inc. website, which list the following products: Lipase PS "Amano," Lipase AH "Amano," Acylase "Amano," Lipase D "Amano," Lipase F-AP 15, Lipase AY "Amano," Lipase M "Amano" 10, Lipase R "Amano," and Lipase G "Amano" 50. Pages taken from the Sanger Institute website and from the German Collection of Microorganisms and Cell Cultures (DSMZ) website, also attached, indicate the equivalence between *Burkholderia cenocepacia* and *Pseudomonas cepacia*, and *Rhizopus oryzae* and strains *R. javanicus* and *R. delamar*, respectively.

Pages printed from the Novozymes A/S website indicate use of the words "Novozym" and "Lipozyme" for commercially available products manufactured by that company. The attached abstract of Yamamoto, et al. recites use of "two lipases[:] Amano PS (*Pseudomonas* sp.) and Novozym 435®," indicating recognized use of these

terms by skilled artisans. Likewise, the attached paper by Maugard and Legroy indicates that "Novozym® SP 435 (lipase from *Candida antarctica* immobilised on an acrylic resin), [and] Lipozyme® (lipase from *Rhizomucor miehei* immobilised on an anionic macroporous resin ...), were [obtained] from Novo Industries (Denmark)." Novo has since moved its industrial enzyme production to Novozymes A/S (*see* printed page from Novozymes referencing the Demerger Document).

Applicants respectfully submit that from these documents, as well as from a quick search of the relevant literature, the examiner may conclude that the introduced trade names are not new matter. To one of skill in the art, familiar with the commercially available enzymes, these additional terms would be inherently present in the table as originally filed.

Likewise, the supplied literature amply supports identification of the relevant enzymes as lipases.

REJECTIONS UNDER 35 USC §112, ¶2

FUNCTIONAL EQUIVALENTS

The entire context in which *E. coli* XL1 Red is discussed in the specification indicates that the relevant function of this strain is mutation. The necessary genetic markers mutS, mutT, and mutD5, now recited in the claims, each create deficiencies in the *E. coli* DNA replication process. Applicants feel that the specification provides ample indication of the relevant functional equivalence for purposes of the presently

claimed process. However, to assuage whatever residual uncertainty remains in the examiner's mind, applicants have indicated this function by appropriate amendment.

ABBREVIATIONS PS AND AH

As indicated above, Lipase PS and Lipase AH are art-recognized portions of trade names under which certain lipases from *Pseudomonas candida* are sold by Amano Enzyme, Inc. The name "Amano" is trademarked in the United States, and as such, should not be used in the claims. The letters PS and AH serve to indicate particular lipase preparations known in the art. From this, and from the above discussion of the relevant literature, what is meant by "*Pseudomonas cepacia* lipase PS" and "*Pseudomonas cepacia* lipase AH" would be abundantly clear to the skilled artisan.

RELA1

Attached to this amendment are copies of an abstract from S. Metzger, et al., (Characterization of the relA1 mutation and a comparison of relA1 with new relA null alleles in Escherichia coli, *J. Biol. Chem.*, 264(35):21146-52 (1989)) and an excerpt from a paper by M. K. B. Berlyn (Linkage Map of *Escherichia coli* K-12, Edition 10: The Traditional Map, *Microbiol. and Mol. Bio. Rev.*, 62(3):814-984 (1998), excerpted pages are 814-822, 834, 856, 866, 885, 888-9, 913, and 948). These documents indicate the level of knowledge held or available to one of skill in the art with reference to the relA1

mutation.

IMPEDING ENZYME ACTIVITY

Applicants have amended the claims to indicate that "a" new catalytic activity is to be introduced into the enzyme, thus making the inherent directionality express. It is respectfully submitted that one of skill in the art would recognize the need or desirability for identifying a specific catalytic activity prior to undertaking the presently claimed process. Once such an inherently necessary step has been performed, both the enzymatic activity which would impede detection and the proper substrate would also be identified.

No limitation is made in claims 12-23 as to the identity of the new catalytic activity to be obtained, or to its relation to the enzyme's original catalytic activity. The new catalytic activity to be obtained may very well be in addition to or in place of that catalytic activity possessed by the identified enzyme prior to performance of the process. The original activity may be maintained, but need not be, necessarily. Such questions would be answerable to one of skill in the art upon performing the particular iteration of the claimed process.

REJECTIONS UNDER 35 USC §112, ¶1

WRITTEN DESCRIPTION - FUNCTIONAL EQUIVALENTS

The examiner states that a "representative number" of species is required to

adequately describe the full scope of functional equivalents. However, "possession" of the invention need not be shown by an actual reduction to practice (*see, e.g., Lockwood v. American Airlines, Inc.*, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997)). Rather, a compound must be defined by "whatever characteristics sufficiently distinguish it" (*Amgen, Inc. v. Chugai Pharmaceutical*, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991)). There is no absolute requirement for description of an actual reduction to practice, so long as one of skill in the art would recognize that the disclosed element is obvious as disclosed, or may immediately envisage the element, relying on knowledge and level of skill in the art (*see, e.g., In re Deuel*, 34 USPQ2d 1210 (Fed. Cir. 1995); *Fujikawa v. Wattanasin*, 39 USPQ2d 1895 (Fed. Cir. 1996); *In re Ruschig*, 154 USPQ 118 (CCPA 1967)).

In the present case, the specification and claims indicate that functional equivalents of the present mutator strain are derivatives of *Escherichia coli* XL1 Red which possess the gene markers relA1, mutS, mutT and mutD5. Production of such derivatives of *E. coli* XL1 Red, given the knowledge and level of skill in the art, would be obvious and straightforward to one of ordinary skill therein. It is respectfully submitted that the present disclosure, coupled with the extensive resources available to the practitioner of genetic recombination, are sufficient to support the claimed range of functional derivatives for purposes of the written description requirement.

ENABLEMENT

Applicants reiterate their belief that the nature of the experimentation required for claims 12-23, though potentially arduous and substantial, would be a matter of routine for the skilled artisan. One of skill in the art would recognize how divergent from an enzyme's original substrate a particular substrate may be to ensure that the new catalytic activity can be produced in that enzyme. The field of enzymology is not so unpredictable that one of skill in the art would not be unable to understand the necessary parameters inherent in practicing the present invention. To answer the examiner's questions, one of skill in the art would apply the knowledge and understanding commonly held with regard to the individual enzyme to be mutated and/or substrate targeted.

CONCLUSION

In view of the foregoing amendments and remarks, applicants consider that the rejections of record have been obviated and respectfully solicit passage of the application to issue. Should the examiner disagree, applicants would sincerely appreciate suggestions as to language and/or subject matter which would be acceptable.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such deposit account.

Respectfully submitted,
KEIL & WEINKAUF

David C. Liechty
Reg. No. 48,692

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Washington, D.C. 20036
(202)659-0100

DCL/kas

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Examples of reaction types that are candidates for enzymes mediation

Application	Products	Form	Opt. pH	Opt. Temp	Speci
Chiral Synthesis	Lipase AS "Amano" *(Aspergillus niger)* Catalogue MSDS APL	Powder	6.0	45°C	12,000-15,000 (Lipolytic activi
	Lipase M "Amano" 10 *(Mucor javanicus)* Catalogue MSDS APL	Powder	7.0	40°C	≧ 10,000 u/g (Lipolytic activi
	Lipase F-AP 15 *(Rhizopus oryzae)* Catalogue MSDS APL	Powder	6.5	40°C	≧ 150,000 u/g (Lipolytic activi
	Lipase G "Amano" 50 *(Penicillium camembertii)* Catalogue MSDS	Powder	5.0	30°C	≧ 50,000 u/g (Lipolytic activi
	Lipase AYS "Amano" *(Candida rugosa)* Catalogue MSDS APL	Powder	7.0	45°C	≧ 30,000 u/g (Lipolytic activi
	Lipase PS "Amano" *(Burkholderia cepacia)* Catalogue MSDS APL	Powder	7.0	50°C	≧ 30,000 u/g (Lipolytic activi
	Lipase AK "Amano" 20 *	Powder	8.0	55°C	≧ 20,000 u/g

	(Pseudomonas fluorescens) Catalogue MSDS APL				(Lipolytic activi
	Cholesterol Esterase "Amano" 2 *(Pseudomonas sp.)* MSDS	Powder lyophilized	7.0	35°C	≧ 10,000 u/g (Lipolytic activi
	Lipase D "Amano" * *(Rhizopus oryzae)* MSDS APL	Powder lyophilized	7.0	50°C	≧ 2,000,000 u/ (Lipolytic activi
	Lipase AH "Amano" * *(Burkholderia cepacia)* MSDS APL	Powder lyophilized	7.0	50°C	≧ 8,000 u/g (Lipolytic activi
LPS	Lipase PS-C "Amano" I * *(Burkholderia cepacia)* Catalogue MSDS Assay Type	Immobilized on ceramic particles	7.0	50°C	≧ 1,000 u/g (Esterification a
	Lipase PS-C "Amano" II * *(Burkholderia cepacia)* Catalogue MSDS Assay Type	Immobilized on ceramic particles	7.0	50°C	≧ 600 u/g (Esterification a
	Lipase PS-D "Amano" I * *(Burkholderia cepacia)* MSDS	Immobilized on diatomaceous earth	7.0	50°C	≧ 500 u/g (Esterification a
	Lipase PS "Amano" *(Burkholderia cepacia)* Catalogue MSDS APL	Powder	7.0	50°C	≧ 30,000 u/g (Lipolytic activi
	LP "Amano" S *(Burkholderia cepacia)* Catalogue MSDS Type APL	Powder lyophilized	7.0	50°C	≧ 2,200,000 u/ (Lipolytic activi
Protease	Protease P "Amano" 10 *(Aspergillus melleus)* Catalogue MSDS APL	Powder	8.0	45°C	≧ 100,000 u/g (Proteolytic act
	Protease N "Amano" *(Bacillus subtilis)*	Powder	7.0	55°C	≧ 150,000 u/g (Proteolytic act

	MSDS				
	Protease S "Amano" *(Bacillus stearothermophilus)* MSDS	Powder	8.0	70°C	≧ 10,000 u/g (Proteolytic act
	Proleather FG *(Bacillus subtilis)* MSDS	Powder	10.0	60°C	≧ 10,000 u/g (Proteolytic act
Acylase	Acylase "Amano" *(Aspergillus sp.)* Catalogue MSDS	Powder	8.5	50°C	≧ 30,000 u/g (pH 8.0)
	D-aminoacylase "Amano" * *(E.coli)* Catalogue MSDS	Powder lyophilized	8.0	40°C	≧ 5,000,000 u/ (pH 8.0)
Other	Glucose Dehydrogenase *(Bacillus sp.)* MSDS	Powder lyophilized	8.0	50°C	≧ 30,000 u/g (pH 8.0)

*under development

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Category	Products	Form	Opt. pH	Opt. Temp	Specification	Applic
Amylase	AMT 1.2L *(Microbacterium imperiale)* Catalogue MSDS	Liquid	6.5	50-55°C	S-Amylase ≧1,200 u/ml (pH 6.0)	Maltotriose Rich
	Transglucosidase L "Amano" *(Aspergillus niger)* Catalogue MSDS	Liquid	5.0	60°C	Transglucosidase ≧300,000 u/ml (pH 5.0)	Isomalto- oligosa Syrup
	Cyclodextrin glucanotransferase "Amano" *(Bacillus macerans)* Catalogue MSDS	Liquid	6.0	60-65°C	CGT-ase ≧600 u/ml (pH 5.5)	Production of cy
Protease	Acid Proteasell *(Rhizopus niveus)* Catalogue MSDS	Powder	3.0	45°C	Protease ≧15,000 u/g (pH 3.0)	Protein Hydrolys
	Acid Protease A *(Aspergillus niger)* Catalogue MSDS	Powder	2.5	55°C	Protease ≧35,000 u/g (pH 3.0)	Protein Hydrolys
	Newlase F *(Rhizopus niveus)* Catalogue MSDS	Powder	3.0	45°C	Protease ≧7,000 u/g (pH 3.0)	Protein Hydrolys
	Protease A "Amano" 2 *(Aspergillus oryzae)* Catalogue MSDS	Powder	7.0	50°C	Protease ≧20,000 u/g (pH 7.0)	Protein Hydrolys
	Protease M "Amano" *(Aspergillus oryzae)* Catalogue MSDS	Powder	4.5	50°C	Protease ≧5,500 u/g (pH 3.0)	Protein Hydrolys

	Protease N "Amano" *(Bacillus subtilis)* Catalogue MSDS	Powder	7.0	55°C	Protease ≧ 150,000 u/g (pH 7.0)	Protein Hydrolys
	Protease NL "Amano" *(Bacillus subtilis)* Catalogue MSDS	Liquid	7.0	55°C	Protease ≧ 20,000 u/ml (pH 7.0)	Protein Hydrolys
	Protease P "Amano" 6 *(Aspergillus melleus)* Catalogue MSDS	Powder	8.0	45°C	Protease ≧ 60,000 u/g (pH 8.0)	Protein Hydrolys
	Protease S "Amano" *(Bacillus stearothermophilus)* Catalogue MSDS	Powder	8.0	70°C	Protease ≧ 10,000 u/g (pH 7.0)	Protein Hydrolys
	Proleather FG-F *(Bacillus subtilis)* Catalogue MSDS	Granule	10.0	60°C	Protease ≧ 10,000 u/g (pH 10.0)	Protein Hydrolys
	Umamizyme *(Aspergillus oryzae)* Catalogue MSDS	Powder	8.0	45°C	Peptidase ≧ 70 u/g (pH 7.0)	Bitterless Proteir
	Peptidase R *(Rhizopus oryzae)* Catalogue MSDS	Powder	7.0	45°C	Peptidase ≧ 420 u/g (pH 7.0)	Bitterless Proteir
Lipase	Lipase A "Amano" 12 *(Aspergillus niger)* Catalogue MSDS	Powder	6.5	45°C	Lipase ≧ 120,000 u/g (pH 6.0)	Fats & Oils Proc
	Lipase AY "Amano" 30 *(Candida rugosa)* Catalogue MSDS	Powder	7.0	45°C	Lipase ≧ 30,000 u/g (pH 7.0)	Fats & Oils Proc
	Lipase F-AP15 *(Rhizopus oryzae)* Catalogue MSDS	Powder	7.0	40°C	Lipase ≧ 150,000 u/g (FIP, pH 7.0)	Fats & Oils Proc
	Lipase G "Amano" 50 *(Penicillium camembertii)* Catalogue MSDS	Powder	5.0	40°C	Lipase ≧ 50,000 u/g (pH 5.6)	Fats & Oils Proc
	Lipase M "Amano" 10 *(Mucor javanicus)* Catalogue MSDS	Powder	7.0	40°C	Lipase ≧ 10,000 u/g (pH 7.0)	Fats & Oils Proc
	Lipase R "Amano" *(Penicillium roqueforti)* Catalogue MSDS	Powder	7.0	30°C	Lipase ≧ 900 u/g (pH 7.0)	Fats & Oils Proc

	Newlase F *(Rhizopus niveus)* Catalogue MSDS	Powder	7.0	40°C	Lipase ≧30,000 u/g (pH 7.0)	Fats & Oils Proc
Yeast Extract	Enzyme RP-1 *(Penicillium citrinum)* Catalogue MSDS	Powder	5.0	70°C	Nuclease ≧13,000 u/mg (pH 4.8)	Production of 5'-
	Deamizyme 50000 *(Aspergillus melleus)* Catalogue MSDS	Powder	5.6	50°C	Deaminase ≧50,000 u/mg (pH 5.6)	Production of Inc
	YL-NL"Amano" *(Bacillus subtilis)* Catalogue MSDS	Liquid	6.0	50°C	Yeast Lysis activity ≧350 u/ml (pH 7.0)	Lyse the yeast c
	Glutaminase Daiwa C100S *(Bacillus subtilis)* Catalogue MSDS	Powder	6.0-7.0	65°C	Glutaminase 110GTU/g±9% (pH 6.0)	Production of L-(
Others	Hyderase *(Aspergillus niger)* Catalogue MSDS	Powder	6.0-8.0	40°C	Glucose oxidase ≧15,000 u/g (pH 7.0)	Removal of Oxy Baking
	Hyderase HC *(Aspergillus niger)* Catalogue MSDS	Powder	6.0-8.0	40°C	Glucose oxidase ≧1,500 u/g (pH 7.0) (High Catalase Content)	Removal of Oxy Baking
	Hyderase L *(Aspergillus niger)* Catalogue MSDS	Liquid	6.0-8.0	40°C	Glucose oxidase ≧10,000 u/ml (pH 7.0)	Removal of Oxy
	Dextranase L "Amano" *(Chaetomium erraticum)* Catalogue MSDS	Liquid	5.5	60°C	Dextranase ≧30,000 u/ml (pH 5.8)	Sugar Refining
	Lactase F "Amano" *(Aspergillus niger)* Catalogue MSDS	Powder	4.5	60°C	Lactase ≧14,000 u/g (pH 4.5)	Lactose Hydroly
	Lactase Y "Amano" L *(Kluveromyces Lactis)* Catalogue MSDS	Liquid	6.5	40°C	Lactase ≧10,000 u/g (pH 6.5)	Lactose Hydroly

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Burkholderia cenocepacia

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The Sanger Institute has been funded by Beowulf Genomics to sequence the genome of *Burkholderia cenocepacia*, in collaboration with Dr. Eshwar Mahenthiralingham of the School of Biosciences, Cardiff University, Prof. John Govan of the Department of Medical Microbiology, University of Edinburgh, Prof. C.A. Hart of the Department of Medical Microbiology, Royal Liverpool Hospital, and Prof. Peter Vandamme of the Laboratorium voor Microbiologie, Universiteit Gent, Belgium.

Burkholderia cenocepacia (formerly *Burkholderia cepacia*, and before that *Pseudomonas cepacia*) is a Gram negative bacterium which is ubiquitous in the environment and may cause a number of diseases in plants. Human infection can be caused by *B. cepacia*, especially in patients with cystic fibrosis and chronic granulomatous disease, and is often fatal. The strain to be sequenced, J2315, is a genomovar III strain of the ET12 lineage, and is the index strain for transmission of this strain among CF patients in the UK. Strain J2315 has been deposited as LMG 16656 in the Belgium Coordinated Collection of Microorganisms.

Note that *B. cenocepacia* J2315 has recently been renamed from *B. cepacia* J2315. Information about this change is published in Vandamme *et al.* (2003) *Res Microbiol.* **154** 91-96

Sequencing is now complete, and the sequences are avalable for searching on our Blast Server, or for download from our FTP site.

The genome totals 8.056 Mb in three chromosomes of 3.870, 3.217 and 0.876 Mb, and a plasmid of 92.7 kb, with a G+C content of approximately 66.9%.

Annotation of the sequence is ongoing, and the full annotation will be released upon publication. Please note that, although the sequence is finished, and we believe it to be accurate, it is possible that errors and missassemblies may remain. The sequence should be considered as preliminary until final publication. We would ask users of the data to read our Data release policy and Guidelines on use of data in publications

This sequencing centre plans on publishing the completed and annotated sequences in a peer-reviewed journal as soon as possible. Permission of the principal investigator should be obtained before publishing analyses of the sequence/open reading frames/genes on a chromosome or genome scale.

Please address all sequencing enquiries to: parkhill@sanger.ac.uk or barrell@sanger.ac.uk

DSMZ - List of Microbial Species: *Rhizopus javanicus* (Filamentous Fungi)

Name	***Rhizopus javanicus*** Takeda, see also: *Rhizopus oryzae* Went & Prinsen Gerligs
Strains	listed under other name: 2192 see: Rhizopus oryzae

DSM 2192 - *Rhizopus oryzae* (Filamentous Fungi)

Name	***Rhizopus oryzae*** Went & Prinsen Gerligs (Filamentous Fungi)
DSM No.	2192
=	CBS 391.34
Information	<- CBS <- R. Nakazawa (*R. javanicus*, type strain). (Medium 129, 30°C)
Medium	129
Supplied as	actively growing culture (on agar or in liquid medium, depending on the strain)
Price	EURO 38 (non-profit making institutions), EURO 54 (other institutions): Normal price.

DSMZ - List of Microbial Species: *Rhizopus delemar* (Filamentous Fungi)

Name	***Rhizopus delemar***
Strains	listed under other name: 853

DSM 853 - *Rhizopus oryzae* (Filamentous Fungi)

Name	***Rhizopus oryzae*** Went & Prinsen Gerligs (Filamentous Fungi)
DSM No.	853
=	ATCC 4858, CBS 327.47
Information	<- K. Kieslich <- ATCC <- A.J. Kluyver (*R. delemar*). Produces pectinase. (Medium 129, 30°C)
Medium	129
Supplied as	actively growing culture (on agar or in liquid medium, depending on the strain)
Price	EURO 38 (non-profit making institutions), EURO 54 (other institutions): Normal price.

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Novozym CALB L	*Candida antarctica* lipase B
Novozym 435	*Candida antarctica* lipase B
Novozym 525 F	*Candida antarctica* lipase B
Lipozyme TL 100 L	Lipase
Lipozyme TL IM	Lipase
Novozym 539 HP F	Protease
Esperase® HP F	Protease
Subtilisin-A	Protease
Esperase HP F	Protease
Cryst. Porcine Trypsin	Trypsin
Novo 4500 K	

Detailed information on these products as well as further informative and supporting literature is available at the Customer Centre at Novozymes.com. If you do not already have access to the Customer Centre, please fill in the application form or contact us.

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Novozym® 435

Novozym 435 is a lipase (lipase B) from *Candida antarctica* produced by submerged fermentation of a genetically modified *Aspergillus* microorganism and adsorbed on a macroporous resin.

Novozym 435 is probably the most commonly used lipase in biocatalysis. It is a highly versatile catalyst with activity towards a great variety of different substrates. The immobilized enzyme is a very thermostable and robust catalyst with activity in a number of organic solvents. It can be used both in batch and column reaction operations but is particularly well suited for use in fixed-bed reactors. Even though Novozym 435 is used in industrial processes as a catalyst in the synthesis of simple esters (including polyesters) and amides and in the regioselective synthesis of carbohydrate monoesters, it is primarily used as a highly enantioselective catalyst in the synthesis of optically active alcohols, amines and carboxylic acids.

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Novozym® 525 F

Novozym 525 F is a lipase (lipase B) from *Candida antarctica* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism. Novozym 525 F is a highly versatile catalyst with activity towards a great variety of different substrates. The enzyme is used in particular as a powerful enantioselective catalyst in the synthesis of optically active alcohols, amines and carboxylic acids.

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Chemo-Enzymatic Syntheses of Both Enantiomers of Neodictyoprolenol and Neodictyoprolene; Possible Biosynthetic Intermediates of Sex Pheromones in Brown Algae

Yuuko Yamamoto, Yoshihiko Akakabe, Kenji Matsui, Hiroshi Shimidzu and Tadahiko Kajiwara*

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* Author for correspondence and reprint requests

Z. Naturforsch. **54c,** 1027–1032 (1999); received June 7/July 12, 1999

Algae, Enantioselective Synthesis, Neodictyoprolene, Pheromones

Neodictyoprolenol [(−)-(*S*)-(1,5*Z*,8*Z*)-undecatrien-3-ol], dictyoprolenol [(−)-(*S*)-(1,5*Z*)-undecadien-3-ol] and their acetates neodictyoprolene [(+)-(*S*)-3-acetoxy-(1,5*Z*,8*Z*)-undecatriene] and dictyoprolene [(+)-(*S*)-3-acetoxy-(1,5*Z*)-undecadiene], which are interesting as possible biosynthetic intermediates of the sex pheromones (dictyopterene **B, C′** and **D′**) of brown algae, were synthesized by chemo-enzymatic methods through optical resolution of racemic neodictyoprolenol and dictyoprolenol using two lipases; Amano PS (*Pseudomonas* sp.) and Novozym 435® (*Candida* sp.). A combination of acylation of the alcohols and hydrolysis of the acetates by Novozym 435® produced neodictyoprolenol, neodictyoprolene, dictyoprolenol and dictyoprolene with high optical purities over 99% enantiomeric excess (*e.e.*).

This snythetic methods will make it easier to search these compounds in marine algae and to study their biosynthesis.

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Enzymatic synthesis of derivatives of vitamin A in organic media

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Abstract

The present article provides an enzymatic method of retinol esterification to reduce photodestruction and irritation problems characteristic of retinol. More specifically, it relates to a method of synthesising retinyl adipate, retinyl succinate, retinyl oleate and retinyl lactate greatly appreciated by cosmetic manufacturer. Desired compounds can be synthesised directly using *Candida antarctica* lipase and *Rhizomucor miehei* lipase in organic media.

Keywords: Retinol; Enzymatic synthesis; Lipase; Cosmetics

1. Introduction

Vitamin A (retinol) and derivatives (retinyl esters, retinoic acid, retinaldehyde), are of great commercial potential in cosmetics and pharmaceuticals such as skin care products. This water-insoluble vitamin has been shown to enhance gap junction communication in a dose-dependent manner, and may promote normal cell growth [1,2]. Vitamin A is necessary for maintenance of healthy epithelial tissue and can prevent the inception or progress of skin cancers by stimulating normal cell differentiation. Deficiency of vitamin A is usually accompanied by a number of severe diseases [3,4]. Consequently, in certain conditions extra amounts are needed in order to cure or prevent certain of these diseases. But vitamin A is very unstable (readily oxidised in air and inactivated by UV light) and difficult to formulate satisfactorily for topical application. Moreover, vitamin A is irritating to the skin. Consequently, to reduce photodestruction and irritation problems, vitamin A may be stabilised by conversion to vitamin A ester (retinyl ester).

Various chemical methods have been published describing the synthesis of retinyl esters. However, retinol degradation often is important, leading to low yields. Moreover, these methods often have the disadvantage that they produce amounts of undesired residual chemical reactants in the final products such as salts.

In view of the attractiveness of retinyl esters as cosmetic agents, as therapeutic and nutritional additives, enzymatic methods can be suggested to produce retinyl esters by a ''biological'' process. Hydrolytic enzymes such as

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lipases in particular, have received special attention because of their effectiveness in regioselective and enantioselective esterifications and transesterifications of organic acids and alcohols in organic solvents [5,6]. Applications of such enzymatic reactions have been considered, for example, in the synthesis of flavor [7–9] and fatty-acid esters [10,11], in the regioselective acylation of carbohydrates [12], in the chemoselective acylation of amino-sugar derivatives [13,14] and in the synthesis of lactones [15], peptides [16], and chiral drugs [17].

The present article presents a preliminary study of the enzymatic retinol esterification using lipases in organic media.

2. Experimental

2.1. Biological and chemical material

Novozym® SP 435 (lipase from *Candida antarctica* immobilised on an acrylic resin), Lipozyme® (lipase from *Rhizomucor miehei* immobilised on an anionic macroporous resin, Duolite 568N), were from Novo Industries (Denmark). Porcine pancreatic lipase, *C. cylindracea* lipase and *Rhizopus arrhizus* lipase, were from Sigma (USA).

The solvents, all analytical grade, retinol, dimethyl adipate and dimethyl succinate, monomethyl succinate, succinic acid, methyl oleate and L-methyl lactate were from Sigma.

2.2. General procedure for the enzymatic reaction

Reactions were conducted in screw-cap glass vials containing 0.5 mmol of retinol **1**, 0.5 mmol of acyl donor, 100 mg of lipase and 0.25 g of 4-Å molecular sieves with 5 ml of solvent. The reaction mixture was stirred under positive argon pressure at 55°C and protected from light. After 50 h the biocatalyst and the molecular sieve were removed by filtration.

2.3. HPLC analysis

Analyses were performed with an HPLC system from Hewlett Packard (processor, pump, UV detector and injector model 1100, differential refractometer (Waters) model 410), equipped with an Ultrasep C18 (250 × 4 mm, 6 μ) reverse phase column from ICS, France. 25 μl of the appropriate dilution of the reaction mixture were injected. A mixture of methanol/water/acetic acid, 90/10/0.3 (v/v/v) was used as eluent at 40°C and a flow rate of 1 ml/min. Products were detected using a UV detector at 280 nm and a differential refractometer. The samples were quantitated by means of calibration curves with pure reagent.

2.4. Purification of reaction products

At the end of the reaction, the biocatalyst was removed by filtration and the solvent evaporated under reduced pressure. The remaining oil was purified by HPLC preparative using an Ultrasep C18 (250 × 8 mm, 6 μ) reverse phase column from ICS, France. A mixture of methanol/water/acetic acid, 90/10/0.3 (v/v/v) was used as eluent at 40°C and a flow rate of 3 ml/min. Products were detected using a UV detector at 280 nm.

2.5. Structural analysis

^{13}C and ^{1}H Nuclear Magnetic Resonance spectra were recorded on a JEOL-JNM LA400 (400 MHz) spectrometer (Laboratoire Commun d'Analyse, Université de La Rochelle), with an internal reference of tetramethylsilane. IR spectra were recorded on a Perkin Elmer Paragon 1000PC instrument in NaCl plate. Mass spectra were recorded on a Varian MAT311 in the "Centre Régional de Mesure Physiques de L'Ouest" (CRMPO), Université de Rennes, France.

(Cad: carbon of adipate; Csu: carbon of succinate; Col: carbon of oleate; Cla: carbon of lactate.)

(Had: proton of adipate; Hsu: proton of succinate; Hol: proton of oleate; Hla: proton of lactate.)

2.5.1. Retinyl methyl succinate (3a)

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1}, and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 173 and 172.5 (2Co*su* ester), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 51.2 (CH_3O), 33.0, 33.3 (CH_2–CO*su*), 23.8 ($2\times CH_2$ *su*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3–C=), 1.89 (3H, s, CH_3–C=), 4.68 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, $2\times$ CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 2.3 (4H, m, 2 CH_2CO*su*), 3.56 (3H, s, OCH_3*su*); $C_{25}H_{36}O_4$ requires M, 400.2613, found M^+, 400.2618; *m/z* 400 (M^+, 3.16%), 268 (M^+–$C_5H_8O_4$, 4.17%), 43 (C_3H_7, 100%).

2.5.2. Retinyl succinate (3b)

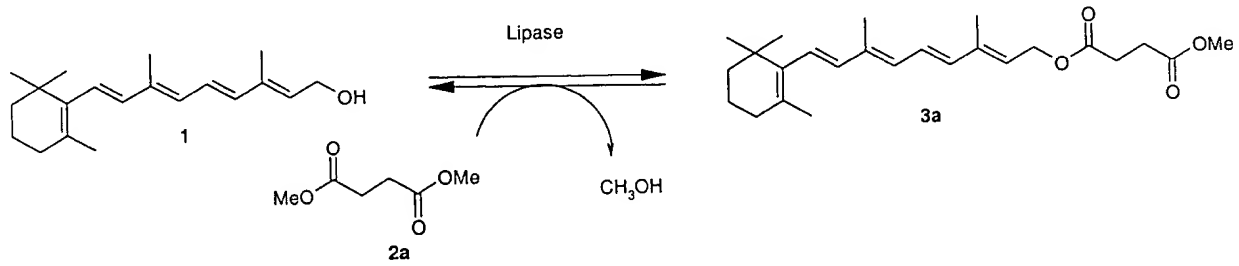
IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1}, ν(CO–O acid) = 1725 cm^{-1} and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 173 (CO*su* ester), 170 (Co*su* acid), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 33.0, 33.3 (CH_2–CO*su*), 23.8 ($2\times CH_2$ *su*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3–C=), 1.89 (3H, s, CH_3–C=), 4.68 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, $2\times$ CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 2.3 (4H, m, 2 CH_2CO*su*); $C_{24}H_{34}O_4$ requires M, 386.2456, found M^+, 386.2462; *m/z* 386 (M^+, 6.32%), 268 (M^+–$C_4H_6O_4$, 5.22%), 43 (C_3H_7, 100%).

2.5.3. Retinyl methyl adipate (3c)

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1} and ν(CO–O ester) = 1737 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 173 and 172.5 (2CO*ad* ester), 138.4, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 51.2 (CH_3O), 33.0, 33.3 (CH_2–CO*ad*), 23.8 ($2\times CH_2$ *ad*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3 to C=), 1.89 (3H, s, CH_3–C=), 4.68 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, $2\times$ CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 1.50 (4H, m, CH_2–CH_2 *ad*), 2.3 (4H, m, 2 CH_2CO*ad*), 3.56 (3H, s, OCH_3*ad*); $C_{27}H_{40}O_4$ requires M, 428.2926, found M^+, 428.2935; *m/z* 428 (M^+, 7.52%), 268 (M^+–$C_7H_{12}O_4$, 3.26%), 43 (C_3H_7, 100%).

2.5.4. Retinyl L-lactate (3d)

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1}, ν(OH) = 3400 cm^{-1} and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 174.5 (CO*la* ester), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.8 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 65.9 (CH*la*), 20.4 (CH_3*la*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3–C=),



Scheme 1.

1.89 (3H, s, CH_3–C=), 4.71 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, 2 × CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 4.13 (1H, m, CH*lac*), 1.23 (3H, d, CH_3*lact*); $C_{23}H_{34}O_3$ requires M, 358.25078, found M^+, 358.2522; *m/z* 358 (M^+, 13.52%), 268 (M^+–$C_3H_6O_3$, 52.87%), 45 ($C_2H_5O_1$, 100%), 43 (C_3H_7, 70.48%).

2.5.5. Retinyl oleate (3e)

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1} and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 174 (CO*ol* ester), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 (2 × CH_3), 12.5 (2 × CH_3 cyclohexenyl), 33.9 (C cyclohexenyl), 130 (2 CH=CH*ol*), 22.7–35.8 (14 CH_2*ol*), 14 (CH_3*ol*); $C_{38}H_{62}O_2$ requires M, 550.4749, found M^+, 550.4754; *m/z* 550 (M^+, 10.86%), 268 (M^+-$C_{18}H_{34}O_2$, 4.17%), 43 (C_3H_7, 100%).

3. Results and discussion

The synthesis of retinyl methyl succinate **3a** from retinol **1** and dimethyl succinate **2a**, was chosen as the model reaction (Scheme 1). The *C. antarctica* lipase (Novozym®) and *R. miehei* lipase (Lipozyme®) were selected as catalysts because these immobilised enzymes are very efficient to the synthesis by reverse hydrolysis in organic media. The reaction was run in screw-cap glass vials containing 0.5 mmol of retinol **1**, 0.5 mmol of dimethyl succinate **2a**, 100 mg of lipase and 0.25 g of 4-Å molecular sieves with 5 ml of different solvents for 50 h at 55°C. From HPLC analysis, the decrease of retinol **1** concentration was seen to be concomitant with the synthesis of **3a**. In the absence of the enzyme, product **3a** did not appear. The

Table 1
Analytical yields of retinyl methyl succinate synthesis in different solvents
All the reactions were carried out at 55°C, with 100 mM of retinol, 100 mM of dimethyl succinate and 10 g/l of lipase in the presence of 4-Å molecular sieves (0.25 g/5 ml solvent). The degree of conversion was determined by HPLC on the base of the substrates' disappearance. No acylation was observed without lipase.

Solvent	Yield (%) with *C. antarctica* lipase	Yield (%) with *R. miehei* lipase	Reaction time (h)
Hexane	75	77	50
4-Methyl-2-pentanone	26	20	50
t-Amyl-alcohol	24	17	50
Dioxane	8	2	50
2-Butanone	0	0	50
Cyclohexanone	0	0	50

Table 2
Esterification of retinol with various acyl donors, catalysed by *R. miehei* lipase
All the reactions were carried out at 55°C, in hexane or in *t*-amyl-alcohol/hexane* (2/3, v/v), with 100 mM of retinol, 100 mM of acyl donor and 10 g/l of lipase in the presence of 4-Å molecular sieves (0.25 g/5 ml solvent). The degree of conversion was determined by HPLC on the base of the substrates' disappearance. No acylation was observed without lipase.

Acylating agent	Solvent	Yield after 50 h (%)	Product
Succinic acid	*t*-Amyl-alcohol/hexane*	4	Retinyl succinate **3b**
Monomethyl succinate	*t*-Amyl-alcohol/hexane*	44	Retinyl methyl succinate **3a**
Dimethyl adipate	Hexane	83	Retinyl methyl adipate **3c**
Methyl lactate	Hexane	86	Retinyl L-lactate **3d**
Methyl oleate	Hexane	90	Retinyl oleate **3e**

isolation, purification and characterisation of product **3a** confirmed that **3a** corresponded to retinyl methyl succinate.

The different yields obtained depending on the nature of solvents and on the nature of lipase, are reported in Table 1. When cyclohexanone and 2-butanone are used as reaction solvents, lipase activity is not detectable. In all the other tests, retinol esterification occurs with variable yields, depending on the solvent and lipase used. The highest yield is obtained in hexane. Three other commercial lipases (porcine pancreatic lipase, *C. cylindracea* lipase and *Rhi. arrhizus* lipase), were tested under the same conditions but no significant reaction was observed.

In addition, using *R. miehei*, other acyl donors were used to produce a wide variety of retinyl esters of great interest in cosmetic formulations. The results are presented in Table 2. Reactions with succinic acid and monomethyl succinate are carried out in *t*-amyl-alcohol/hexane system (2/3, v/v), in order to solubilise acyl donors which are insoluble in hexane. With the exception of monomethyl succinate, we observed excellent yields (more than 80%) for all the methyl ester donors. On the other hand, with succinic acid and monomethyl succinate, we found a lower reactivity than with the methyl ester donors.

In order to improve the system performance and to shift the reaction equilibrium towards synthesis, all the reactions were run in *t*-amyl-alcohol at 90°C under reduced pressure. The co-produced methanol was then removed more rapidly than under atmospheric pressure. We used *C. antarctica* lipase. This one has already shown its efficiency in similar conditions. However, in these conditions, the retinol degradation increases more rapidly than the retinol acylation. Indeed, retinol methyl succinate formation is competitive with the destruction of retinol characterised by the production of several compounds more hydrophilic than retinol.

4. Conclusion

An efficient method has been developed for the lipase-catalysed synthesis of retinyl esters such as retinyl succinate, retinyl adipate, retinyl oleate and retinyl lactate. To our knowledge, this enzymatic process is the first that describes retinyl esters synthesis with such excellent yields. In addition, the retinyl lactate produced may be considered as an excellent carrier of lactic acid. Indeed, in the presence of esterase-type epidermal enzymes, they can undergo hydrolysis, thus releasing lactic acid progressively. Derived from fruit and dairy products, alpha hydroxy acids are widely included in cosmetics as exfoliants. The most commonly used are lactic acid and glycolic acid, both of which seem to exert slight but significant effects in reducing skin discolorations and roughness when applied in a cream [18,19]. Significant irritation is often associated with the use of alpha hydroxy acids alone. Esters of retinol and alpha

hydroxy acids are unusually effective as skin conditioners, with significant reductions in the irritation problems characteristic of retinol and alpha hydroxy acids in nonesterified form [20].

Acknowledgements

The generous gift of *C. antarctica* lipase and *R. miehei* lipase by Novo-Nordisk is gratefully acknowledged.

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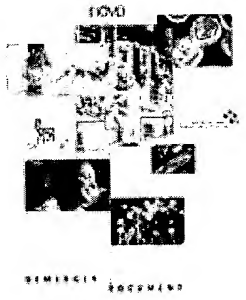

In February 1999, Novo Nordisk announced its plans to demerge the company's activities within health care and enzymes for industrial use into separately listed legal entities.

The decision was taken at an extraordinary general meeting on 13 November 2000 in Copenhagen, Denmark. The demerger took effect retroactively as of 1 January 2000.

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1: J Biol Chem. 1989 Dec 15;264(35):21146-52.

Characterization of the relA1 mutation and a comparison of relA1 with new relA null alleles in Escherichia coli.

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The most widely studied "relaxed" mutant of the relA locus, the relA1 allele, is shown here to consist of an IS2 insertion between the 85th and 86th codons of the otherwise wild-type relA structural gene, which normally encodes a 743-amino acid (84 kDa) protein. The RelA protein is a ribosome-dependent ATP:GTP (GDP) pyrophosphoryltransferase that is activated during the stringent response to amino acid starvation and thereby occasions the accumulation of guanosine 3',5'-bispyrophosphate (ppGpp). We propose that the IS2 insertion functionally splits the RelA protein into two (alpha and beta) peptide fragments which can complement each other in trans to yield residual ppGpp synthetic activity; neither fragment shows this activity when expressed alone. Cell strains with a single copy relA null allele show physiological behavior that is much the same as relA1 mutant strains. Both relA1 and relA null strains accumulate ppGpp during glucose starvation and do not accumulate ppGpp during the stringent response. The presence of ppGpp in verifiable relA null strains is interpreted as unequivocal evidence for an alternate route of ppGpp synthesis that exists in addition to the relA-dependent reaction.

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Linkage Map of *Escherichia coli* K-12, Edition 10: The Traditional Map

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INTRODUCTION

Previously, Berlyn et al. (323) presented the traditional map, the EcoMap physical map, and a map by Singer and Low showing the distribution of the Gross-Singer transposon set around the chromosome. The map in this paper is a revision of that traditional map of *Escherichia coli* K-12, the linkage map of known genes and other functional sites (Fig. 1), and the physical map, EcoMap 10, of Kenneth Rudd is presented in the companion article (3763a).

The linkage map in this presentation includes genes located primarily by restriction, sequence, and cotransduction data reported in the literature and databases. It uses coordinates based on the complete sequence released by the Blattner laboratory. Obviously, the sequence is now the major resource for placing genes on the map. In some regions the placement represents a shift from the edition 9 map, which was based on coordinates of Rudd's EcoMap 7 composite of sequenced genes and regions (27, 33, 395, 568, 569, 926, 3308, 3465, 4127, 4128), placed on the physical map of *Escherichia coli* (2291, 3763b) by restriction and sequence comparisons. Those map positions were based on the results in the literature and on EcoMap and GenBank database entries. EcoMap 10 coordinates are of course also based on the completed sequence, and cross-consulting this summary map and the EcoMap that follows should be straightforward.

The linkage map of Fig. 1 includes 2,220 genes and about 40 other chromosomal markers, such as phage attachment sites, defective-phage elements, replication origins and termini, and other features traditionally included on the published linkage map. It does not include open reading frames (ORFs) lacking evidence for expression, with unknown functions or putative functions inferred by sequence homologies only. A few exceptions occur for *Salmonella* genes where the inference is strong that they are also expressed in *E. coli*. The ORFs not included in this map can be found on EcoMap 10. The Fig. 1 map places the genes that can be found in sequence annotation and EcoMap 10 on the right side of the line. On the left side are genes not present on physical maps or the sequence, and in most cases these are not connected to a specific point on the axis to indicate that the localization is only approximate. As in previous editions of the *E. coli* linkage map (187, 188, 189, 190, 190a, 323, 4368, 4369, 4370, 4371), an asterisk indicates that the gene is not precisely located with respect to near neighbors and parentheses indicate that the location is even more uncertain and that the gene is located only within that general region. I have been very conservative about removing these from the map; even though the usefulness of some of these may be quite limited, there will probably be cases where the old, sometimes poorly characterized phenotype may be helpful in ascribing functions and phenotypic effects to ORFs. Also shown on the left side in boldface followed by colons are operon names that are distinct from any gene name within the operon and termination and attachment sites. The arrows indicate the direction of transcription and span genes included within a transcription unit.

Updates of map information are available in electronic form from several sites. These include the *E. coli* Genetic Stock Center's (CGSC's) World Wide Web server at URL http://cgsc.biology.yale.edu, which provides an interface for querying the database and retrieving formatted reports about genes, map regions, strains, and mutations, etc. (323a); the National Center for Biotechnology Information ftp site for EcoSeq and EcoMap, ncbi.nlm.nih.gov/repository/Eco/EcoMap7; the Colibri map at http://www.pasteur.fr/Bio/Colibri.html, the ECDC map at http://susi.bio.unigiessen.de/ecdc.html, the site for the sequencing project at the University of Wisconsin, http://www.genetics.wisc.edu, a gene-protein database, http://www.mbl.edu/html/ecoli.html, Genome Information Broker at http://mol.genes.nig.ac.jp/ecoli, and others. See also Rudd (3673). The references attempt to document map information, the basic definition of the gene's function, and expression information and do not include information relating to detailed physical structure, active site in vitro mutagenesis, or enzyme

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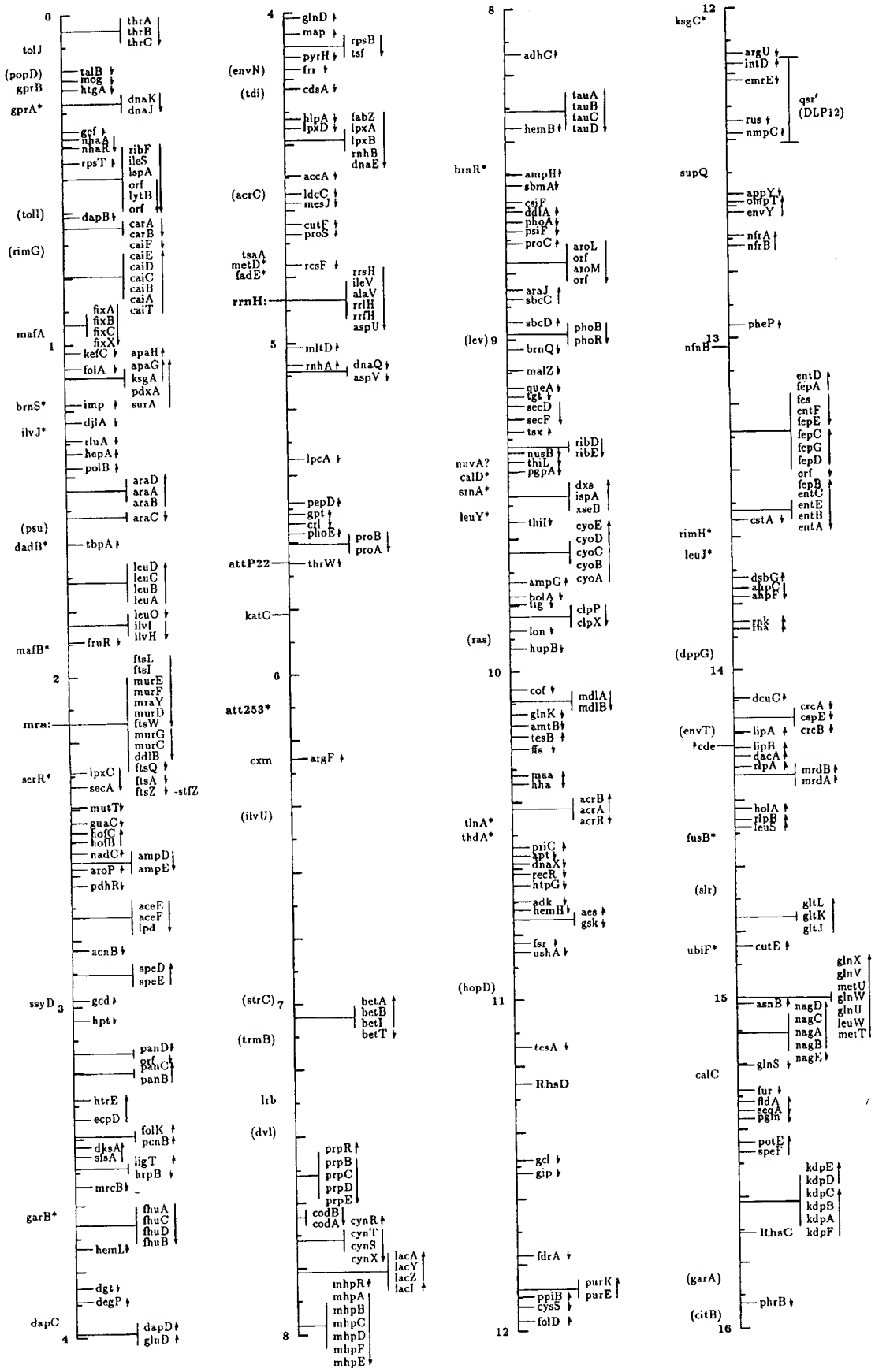
FIG. 1. Linear drawing of circular linkage map of *E. coli* K-12. Symbols are defined in Table 1. Arrows show the direction of transcription. Where T-bars are used to display groups of genes, the length of the T shows the approximate length and position of the group in terms of the map coordinates, allowing visual ordering of closely packed groups.


0
thrA
thrB
thrC
tolJ
(popD)
talB
mog
gprB
htgA
dnaK
gprA*
dnaJ
gef
nhaA
nhaR
ribF
rpsT
ileS
lspA
orf
lytB
orf
(tolI)
dapB
carA
carB
(rimG)
caiF
caiE
caiD
caiC
caiB
caiA
caiT
fixA
fixB
fixC
fixX
mafA
1
kefC
apaH
folA
apaG
ksgA
pdxA
brnS*
imp
surA
djlA
ilvJ*
rluA
hepA
polB
araD
araA
araB
araC
(psu)
dadB*
tbpA
leuD
leuC
leuB
leuA
leuO
ilvI
ilvH
mafB*
fruR
ftsL
ftsI
2
murE
murF
mraY
murD
mra:
ftsW
murG
murC
ddlB
ftsQ
lpxC
ftsA
serR*
secA
ftsZ
-stfZ
mutT
guaC
hofC
hofB
nadC
ampD
aroP
ampE
pdhR
aceE
aceF
lpd
acnB
speD
speE
ssyD
3
gcd
hpt
panD
grf
panC
panB
htrE
ecpD
folK
pcnB
dksA
sfsA
ligT
hrpB
mrcB
garB*
fhuA
fhuC
fhuD
fhuB
hemL
dgt
degP
dapC
4
dapD
glnD
4
glnD
map
rpsB
tsf
pyrH
frr
(envN)
cdsA
(tdi)
hlpA
fabZ
lpxD
lpxA
lpxB
rnhB
dnaE
accA
(acrC)
ldcC
mesJ
cutF
proS
tsaA
metD*
fadE*
rcsF
rrsH
ileV
alaV
rrlH
rrfH
aspU
rrnH:
5
mltD
rnhA
dnaQ
aspV
lpcA
pepD
gpt
ctl
phoE
proB
proA
attP22
thrW
katC
6
att253*
cxm
argF
(ilvU)
(strC)
7
betA
betB
betI
betT
(trmB)
lrb
(dvl)
prpR
prpB
prpC
prpD
prpE
codB
codA
cynR
cynT
cynS
cynX
lacA
lacY
lacZ
lacI
mhpR
mhpA
mhpB
mhpC
mhpD
mhpF
mhpE
8
8
adhC
tauA
tauB
tauC
tauD
hemB
brnR*
ampH
sbmA
csiF
ddlA
phoA
psiF
proC
aroL
orf
aroM
orf
araJ
sbcC
sbcD
phoB
phoR
(lev) 9
brnQ
malZ
queA
tgt
secD
secF
tsx
ribD
ribE
nusB
thiL
pgpA
nuvA?
calD*
srnA*
dxs
ispA
xseB
leuY*
thiI
cyoE
cyoD
cyoC
cyoB
cyoA
ampG
holA
tig
clpP
clpX
(ras)
lon
hupB
10
cof
mdlA
mdlB
glnK
amtB
tesB
ffs
maa
hha
acrB
acrA
acrR
tlnA*
thdA*
priC
apt
dnaX
recR
htpG
adk
hemH
aes
gsk
fsr
ushA
(hopD)
11
tesA
RhsD
gcl
gip
fdrA
purK
purE
ppiB
cysS
folD
12
12
ksgC*
argU
intD
emrE
qsr'
(DLP12)
rus
nmpC
supQ
appY
ompT
envY
nfrA
nfrB
pheP
nfnB
13
entD
fepA
fes
entF
fepE
fepC
fepG
fepD
orf
fepB
entC
entE
entB
entA
cstA
rimH*
leuJ*
dsbG
ahpC
ahpF
frk
rha
(dppG)
14
dcuC
crcA
cspE
crcB
(envT)
lipA
cdc
lipB
dacA
rlpA
mrdB
mrdA
holA
rlpB
leuS
fusB*
(slr)
gltL
gltK
gltJ
ubiF*
cutE
glnX
glnV
metU
15
asnB
glnW
nagD
glnU
nagC
leuW
nagA
metT
nagB
nagE
glnS
calC
fur
fldA
seqA
pgm
potE
speF
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kdpA
RhsC
kdpF
(garA)
phrB
(citB)
16

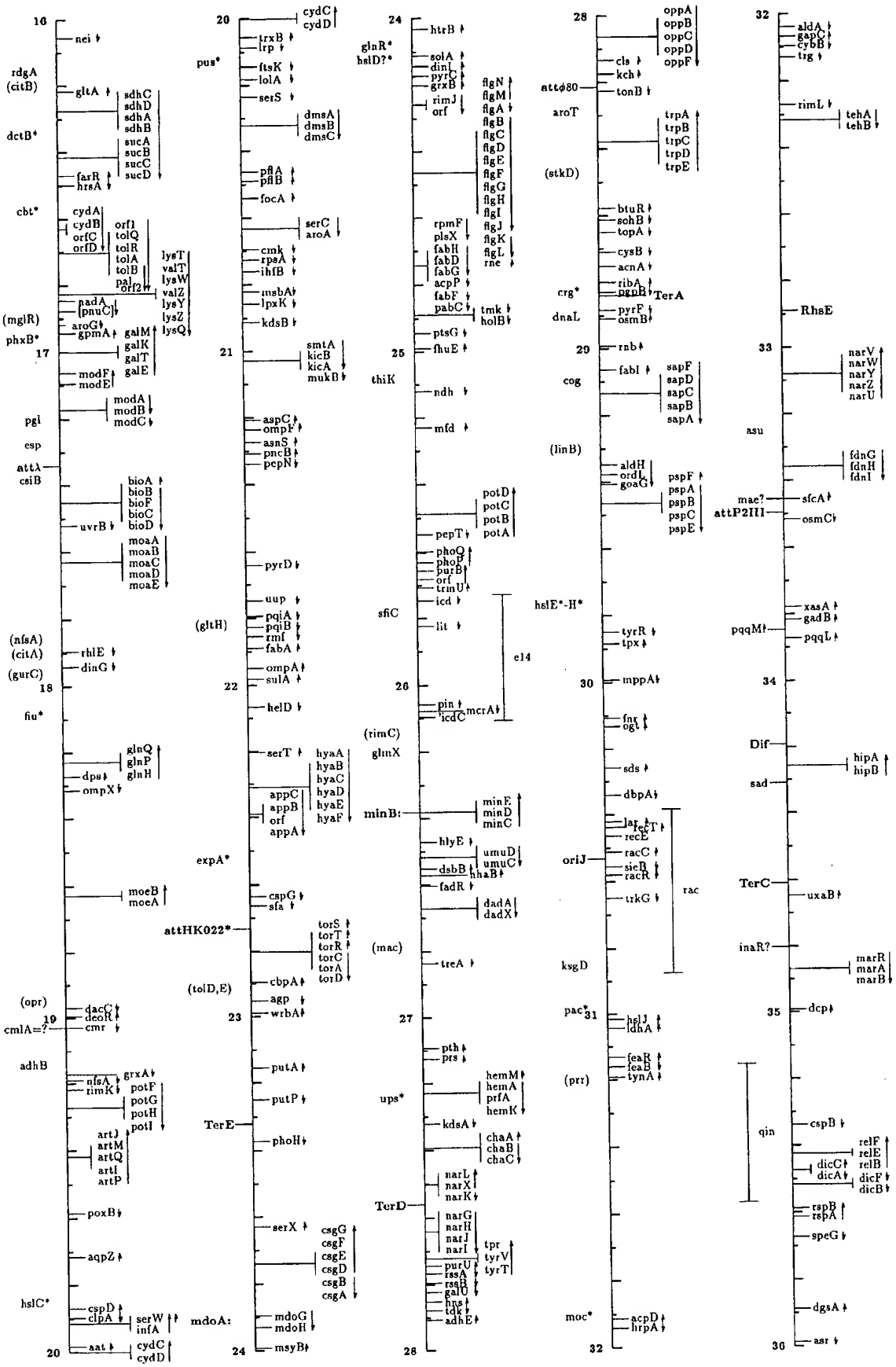


FIG. 1—*Continued.*

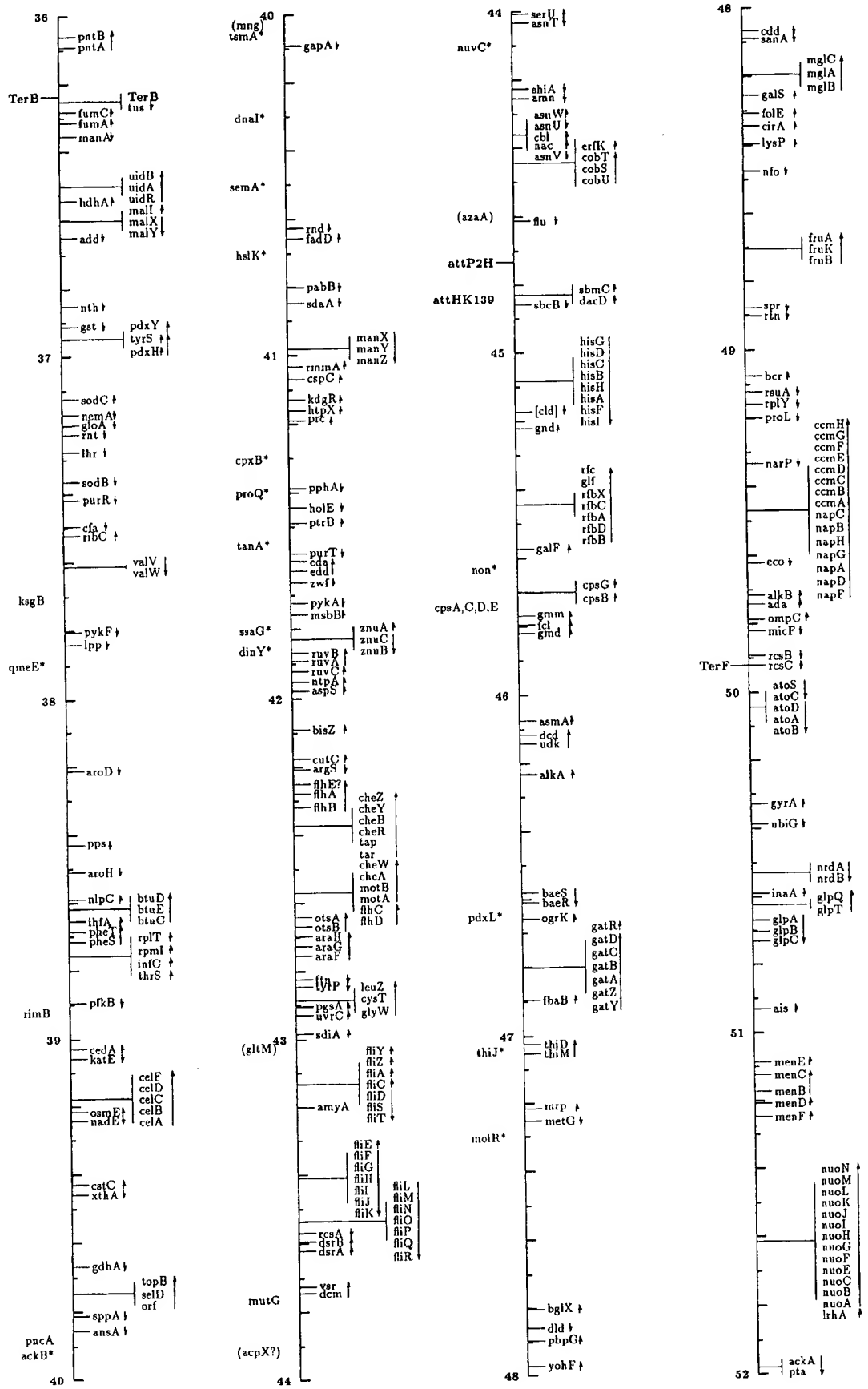
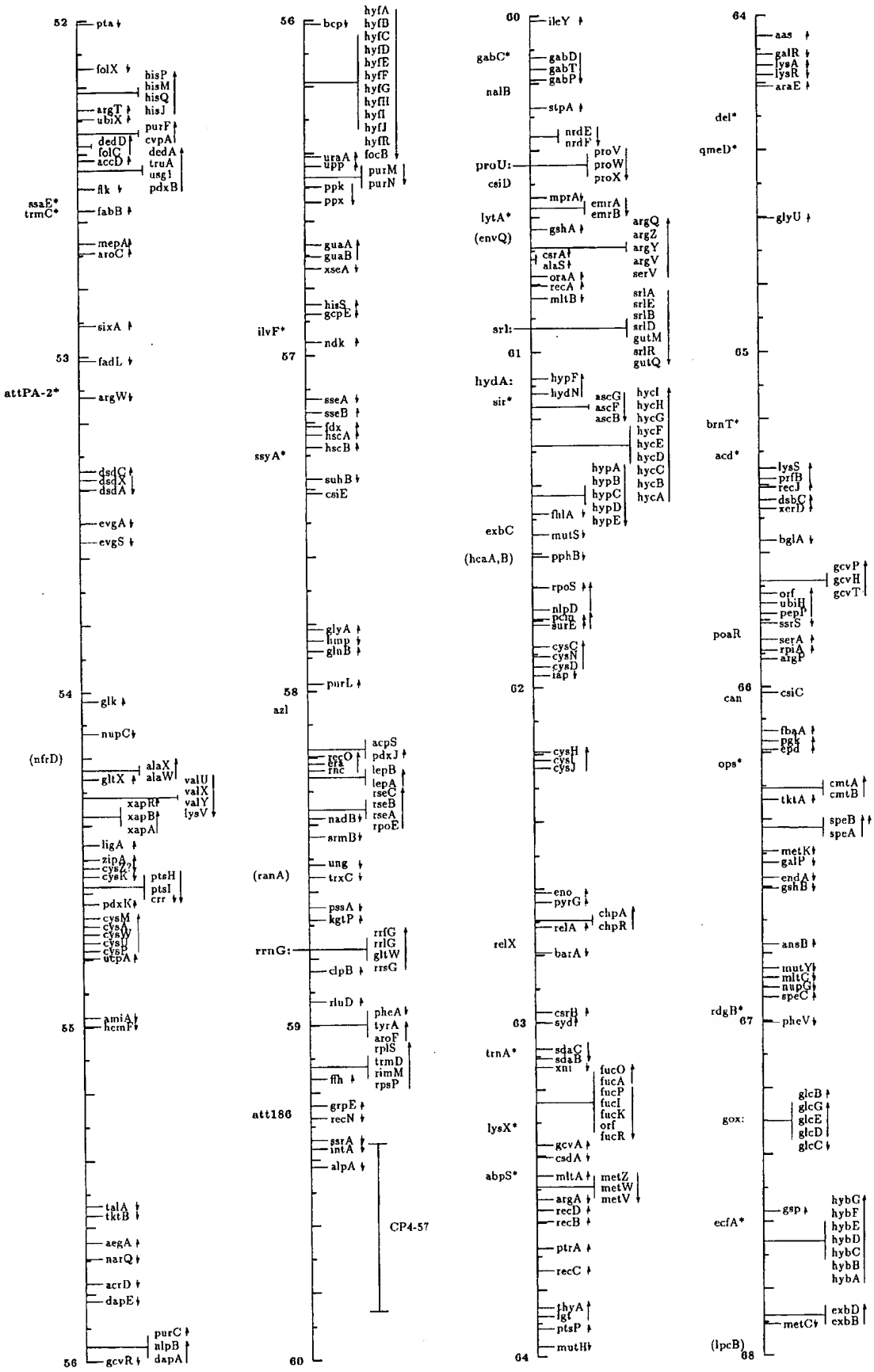


FIG. 1—*Continued.*



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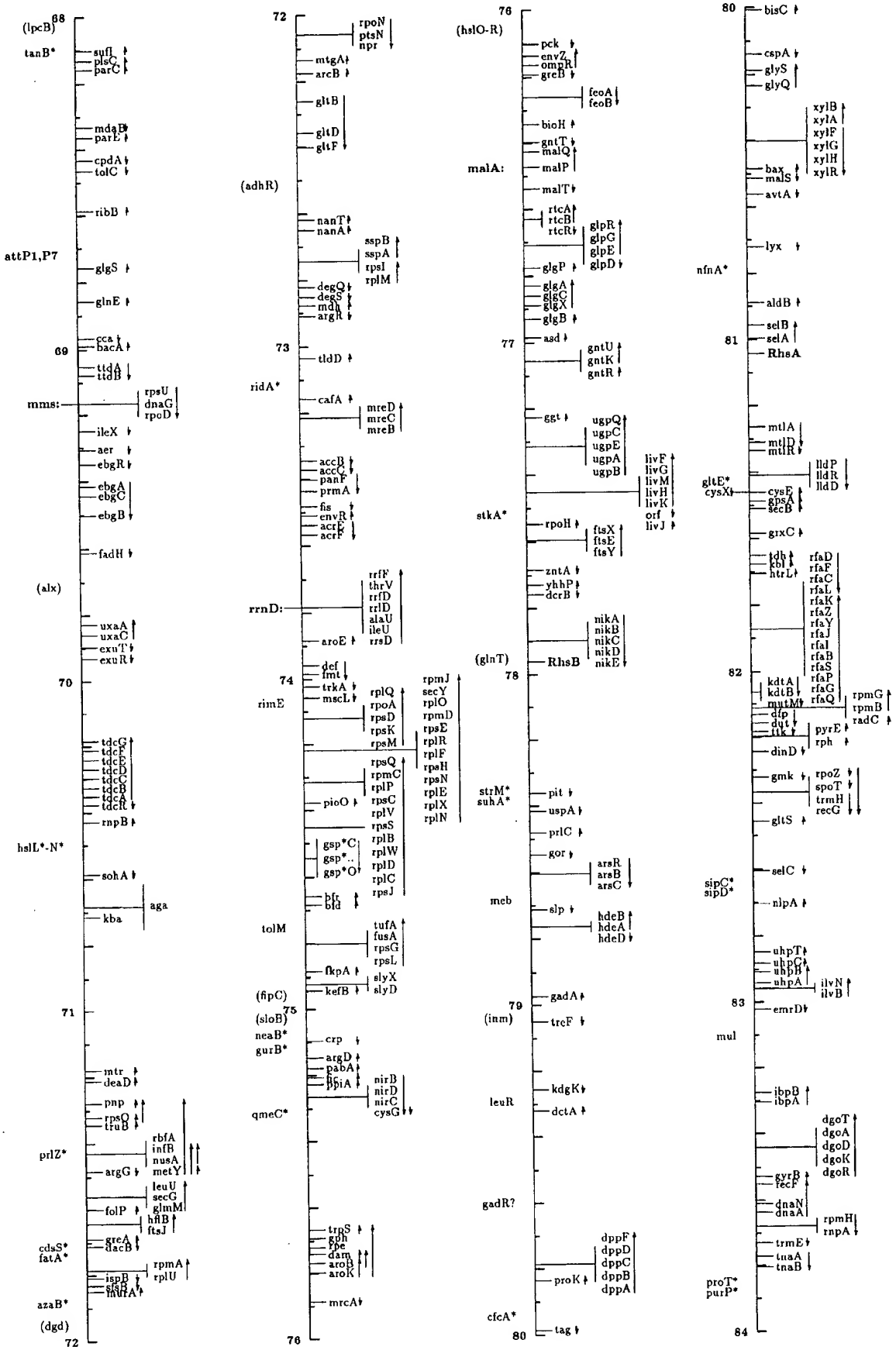

FIG. 1—*Continued.*

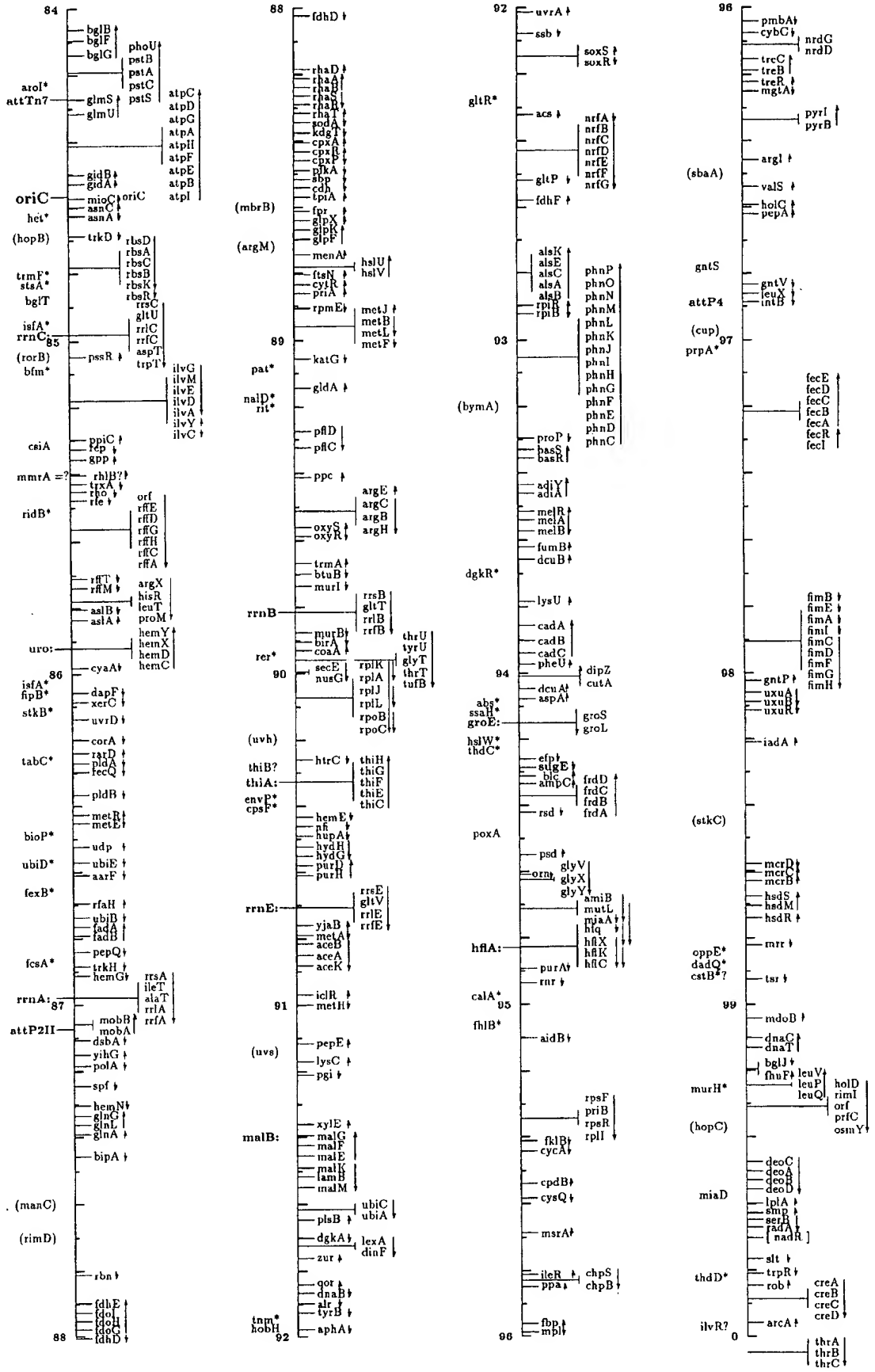

FIG. 1—*Continued.*

mechanism. Earlier map papers contain additional references for some of the loci (188–190a, 323).

MAP UNITS

Since the 1976 recalibration of the linkage map in terms of minutes required for time of entry of markers in interrupted conjugation experiments, the standard representation of the map has used the basic units of minutes and a total length of 100 minutes (190a). This has been a convenient and accepted coordinate system for the map, and although the current map units are based on restriction and sequence data rather than time of entry, we retain the term minute for 1/100 of the length of the chromosome. Both the CGSC database and EcoMap use as "left endpoints" the counterclockwise boundary of the coding region, and genes in Fig. 1 are placed approximately at these coordinates, with the higher-resolution map of Rudd (3763a) providing more exact placement, showing nucleotide and minute coordinates for the physically mapped genes.

NOMENCLATURE

Gene Symbol Convention

The standard genetic nomenclature for *E. coli* is that of Demerec et al. (1016), as subsequently amended through use, and as described in Instructions to Authors for the *Journal of Bacteriology* (see also reference 3821). This map, like those preceding it, follows those nomenclatural conventions. Accordingly, we have adhered to a three-letter lowercase mnemonic symbol, with an uppercase letter added when there are two or more genes in that mnemonic category. If authors have added an uppercase letter for a gene in a single-instance category, we have used that published four-letter symbol. For attachment sites and noncoding features of the chromosome, etc., the same standard has not been used, and we have continued to use the variable-length symbols historically applied to these sites. We have continued the convention proposed for sites of termination of replication and repetitive sequences, by using italicized symbols with the first letter uppercase.

The Issue of Stability

Many names have been changed by investigators since the 1990 map was published. When those changes were part of a systematic revision of nomenclature (often aimed at clarifying usage and resolving conflicts) for a group of related genes and were in compliance with the current *E. coli* gene nomenclature system, or were changed for compelling mnemonic reasons or for resolution of redundancy or conflict, also in conformance with the standard system, we have adopted those changes. We have not adopted and we wish to discourage changes of valid preexisting names proposed by authors simply because they believe that theirs is a symbol signifying a more apt or accurate mnemonic. For example, a previously published name based on the pathway or phenotype is valid and should not, simply as a matter of course, be replaced by an alternate mnemonic based on the name of the enzyme that the gene codes for once that functional information has been determined. In general, the stability of a name has more value than improved nuances. In a few cases, we have been compelled to use a new name, despite the apparent validity of the original name, simply because the new name has been widely adopted in the literature. In a number of cases, a new gene has been assigned a symbol which has already been used or which is simultaneously proposed for another gene, with the two mnemonics having entirely different meanings. These names have had to be resolved, usually by changing the newer assignment. In a few cases, a uniquely named gene has been shown later to belong to a category for which a symbol already exists, and the latter symbol has been used instead of the earlier assignment. There is one case in this paper where use of a symbol already assigned to a different gene was strongly preferred by authors, and I was very reluctant to suggest a new symbol for the earlier, published gene name to the earlier authors, since that symbol has been used in a number of publications; for the interim I have broken convention to assign the newer genes temporary symbols with asterisks (*gsp**), in order to show them on the map and in the hope of resolving that naming with the usual precedence custom in the near future. Some synonymy is unavoidable, since a gene under study may be named and described in print before its identity to a known gene is discovered. However, a common practice in the recent literature seems to allow publication of an author's preliminary name for a gene even if its identity to a known gene has been discovered before publication, and that practice creates unnecessary synonymy. Alternate gene symbols are listed in Table 1, and Table 2 provides an alphabetized list of such symbols with cross-references to the symbols used in Table 1.

There is a standing tradition of coordinating gene symbols between the CGSC and the *Salmonella* Genetic Stock Center to avoid the assignment of the same symbol to different genes in the two organisms and the assignment of different symbols to homologous genes, insofar as this coordination is feasible. We have not, however, changed names of *E. coli* genes in order to extend this tradition to other bacteria or other organisms. The desirability and feasibility of uniform nomenclature conventions for all bacteria or other microbial groupings are currently only topics of discussion and conjecture, and changes to enhance similarities in an ad hoc, piecemeal fashion seem counterproductive at the present time. Readers are reminded that symbol changes create discontinuity with previous literature concerning genes, with even more serious ramifications for allele designations, since unique allele numbers are assigned on the basis of the three letter mnemonic and changes in a symbol may necessitate renumbering of alleles as well.

TABLE 1. *E. coli* genes and replication- or phage-related sites[a]

Gene symbol	Map location (min)	Mnemonic for symbol	Synonyms and gene product—enzyme, RNA, or phenotype affected	CGSC no.[d]	References[e]
aarF	86.6	Aminoglycoside acetyltransferase regulator	*yigQR*; regulator of 2′-*N*-acetyltransferase; involved in respiratory cofactor ubiquinone production	53879	2696
aas	64.1	Acyl-ACP synthase	2-Acyl-glycerophosphoethanolamine acyltransferase; acyl-ACP synthetase; salvage pathway for reacylation; inner membrane; bifunctional for turnover/incorporation	29780	1831, 1972
aat	20.0	Amino acyl-tRNA-protein transferase	Aminoacyl-tRNA-protein-transferase (EC 2.3.2.6)	1054	4045
abpS	63.5	Arg binding protein	Low-affinity transport system for arginine and ornithine; periplasmic binding protein	18562	664
abs	94.1	Antibiotic sensitivity	Sensitivity and permeability to antibiotics and dyes	18559	763
accA	4.5	Acetyl-CoA carboxylase	Acetyl-CoA carboxylase α-carboxyltransferase subunit: (EC 6.4.1.2)	29829	2536, 2537
accB	73.4	Acetyl-CoA carboxylase	*fabE*; acetyl-CoA carboxylase, biotin carboxyl carrier protein (EC 6.4.1.2)	796	2537, 2712, 3057, 4302, 4616
accC	73.4	Acetyl-CoA carboxylase	*fabG*; acetyl-CoA carboxylase, biotin carboxylase (BC) subunit (EC 6.4.1.2)	29834	2315, 2537, 3253
accD	52.4	Acetyl-CoA carboxylase	*dedB*, *usg*; acetyl-CoA carboxylase β-carboxyltransferase subunit (EC 6.4.1.2)	28570	2534, 2537, 3081
acd	65.1	Acetaldehyde-CoA deHase	Acetaldehyde-CoA dehydrogenase (EC 1.2.1.10)	1053	764
aceA	90.8	Acetate	*icl*; isocitrate lyase (EC 4.1.3.1); acetate utilization	1052	2744, 2811, 3674, 754, 841
aceB	90.8	Acetate	*mas*; malate synthase A (EC 4.1.3.2)	1051	2744, 591, 592, 840, 841
aceE	2.7	Acetate	*aceE1*; pyruvate dehydrogenase (decarboxylase component) E1p; (EC 1.2.4.1); acetate requirement	1050	1553, 1555, 1556, 1558, 2934, 4150, 4204, 655
aceF	2.7	Acetate	*aceE2*; pyruvate dehydrogenase (dihydrolipoyltransacetylase component) E2p (EC 1.6.4.3, EC 2.3.1.12); acetate requirement	26530	1554, 2934, 655
aceK	90.9	Acetate	Isocitrate dehydrogenase kinase/phosphatase	17770	1343, 1897, 2274, 2418, 2419, 753, 842, 754
ackA	52.0	Acetate kinase	Acetate kinase (EC 2.7.2.1); mutants fluoroacetate resistant	1048	1548, 2094, 2512, 2812, 530, 224
ackB	39.9	Acetate kinase	Acetate kinase activity[x] (EC 2.7.2.1)	1047	3379
acnA	28.8	Aconitase	Aconitase A (EC 4.2.1.2)	28218	3542, 3543
acnB	2.8	Aconitase	Aconitase B (EC 4.2.1.2)	36955	1538, 2141, 474
acpD	31.9	Acyl carrier protein	ACP phosphodiesterase	52896	1256, 394
acpP	24.8	Acyl carrier protein	ACP (acyl carrier protein)	31871	2183, 3621
acpS	58.2	Acyl carrier protein	*dpj*; holo-ACP synthase (EC 2.7.8.7)	32953	2397, 2400, 2401, 3471, 4340
acpX	43.9	Acyl carrier protein	*acpS*; originally thought to be holo-ACP synthase; perhaps cryptic second gene or regulator	1046	3471
acrA	10.4	Acridine	*Mb*, *lir*, *mbl*, *mtcA*, *sipB*; AcrAB efflux system effects Mar multiple resistance	1045	1291, 1726, 2684, 2685, 3282, 3563, 4723, 808
acrB	10.4	Acridine	*acrE*; AcrAB system has major role in Mar multiple resistance to NAL, TET, AMP, etc.; beware renamings of *acrE*, *acrB*, and *envC*	35806	1291, 2684, 2685, 3282, 4723
acrC	4.5	Acridine	Sensitivity to acriflavine; transmembrane protein	1044	3104
acrD	55.7	Acridine	Sensitivity to acriflavine	35697	3188
acrE	73.5	Acridine	*envC*; anomolous cell division; chain formation; splits cross-wall to form new poles; see *acrB*	813	2133, 2255, 2256, 2516, 3702
acrF	73.6	Acridine	*envD*; encodes lipoprotein with signal peptide; osmotically remedial envelope defect	33608	2255, 2256, 2516, 2684
acrR	10.5	Acridine	Regulatory protein for *acrA* and *acrB*	35809	2686
acs	92.3	Acetyl-CoA synthetase	Acetyl CoA synthetase 2 (EC 6.2.1.1)	34317	2367, 395
ada	49.7	Adaptive (response)	O^6-methylguanine-DNA methyltransferase, inducible; DNA repair against methylating and alkylating agents; transcription factor	1043	1184, 2001, 2158, 2410, 2489, 2698, 2760, 2967, 3078, 3094, 3795, 3812, 3815, 3957, 3958, 3959, 4330, 4385
add	36.6	Adenine deaminase	Adenosine deaminase (EC 3.5.4.4.); mutants affect growth on deoxyadenosine in *purA*, *B* mutants	1042	2024, 765

Continued on following page

TABLE 1—*Continued*

Gene symbol	Map location (min)	Mnemonic for symbol	Synonyms and gene product—enzyme, RNA, or phenotype affected	CGSC no.[d]	References[e]
dinG	17.9	Damage inducible	LexA regulated (SOS) repair enzyme	31247	2318, 2518, 2519
dinI	24.1	Damage inducible	Multicopy suppresses phenotype of cold-sensitive *dinD* filamentous mutation	53428	4877
dinY	41.9	Damage inducible	Repair gene	36880	3412
dipZ	94.0	Disulfide isomerase	*cycZ*, *dsbD*, *cutA2*; may be involved in cytochrome maturation, see *ccm* genes; affects disulfide binding	34213	1921, 268, 2910, 874, 875
djlA	1.2	DnaJ-like	Proposed to dock and interact with variety of membrane proteins; mutants rapidly accumulate suppressors	51192	2188, 769, 770
dksA	3.5	*dnaK* suppressor	*msmA*; high copy suppresses *muk* and TS growth and filamentation of *dnaK* mutant	30521	2122, 4842
dld	47.9	D-Lactate dehydrogenase	*ldh*; D-lactate dehydrogenase (EC 1.1.1.28); vinylglycolate resistance, FAD enzyme	852	2058, 3768, 4012, 626
dmsA	20.3	DMSO reductase	DMSO reductase subunit A, anaerobic	17710	353, 354, 3749
dmsB	20.3	DMSO reductase	DMSO reductase subunit B; apparent Fe-S binding domain; anaerobic	31724	354, 3749
dmsC	20.3	DMSO reductase	DMSO reductase subunit C, membrane bound	31727	354, 3749
dnaA	83.6	DNA	DNA biosynthesis; initiation; binding protein	851	1372, 1630, 1631, 1872, 1873, 1875, 2160, 2235, 2272, 2275, 2474, 2927, 2985, 2986, 3051, 3261, 3400, 3541, 3569, 3570, 3809, 3813, 4087, 4300, 4301, 4611, 471, 4910, 1790
dnaB	91.9	DNA	*groP*, *grpA*, *grpD*; DNA biosynthesis; chain elongation	850	1406, 2259, 2545, 3126, 3333, 3804, 3951, 4449, 561
dnaC	99.1	DNA	*dnaD*: DNA biosynthesis; initiation and chain elongation	849	2786, 3125, 3753
dnaE	4.4	DNA	*polC*, *sdgC* (suppressor of *dnaG* mutation); DNA polymerase III, α-subunit	373	1239, 1240, 2194, 296, 3889, 4016, 4424, 4708, 4761
dnaG	69.2	DNA	*dnaP*, *parB*, *sdgA*; primase; primer synthesis for leading- and lagging-strand synthesis	847	1531, 2671, 2672, 2673, 3055, 3113, 3125, 3216, 3753, 4105, 4376, 4449, 4578, 4766, 579
dnaI	40.3	DNA	DNA biosynthesis	846	334a
dnaJ	0.3	DNA	*groP*, *grpC*; chain elongation; stress-related DNA biosynthesis, responsive to heat shock; chaperone with DnaK	845	1406, 2069, 231, 3256, 3257, 3258, 3333, 3804, 4252, 4293, 4830, 972
dnaK	0.3	DNA	*gro*, *groP*, *groPAB*, *groPC*, *groPF*, *grpC*, *grpF*, *seg*; stress-related heat-shock DNA biosynthesis, ATP-regulated binding and release of polypeptide substrates; HSP-70-type molecular chaperone, with DnaJ	844	1143, 1186, 1187, 1875, 2120, 227, 2837, 3035, 3158, 3257, 3332, 3807, 4252, 567, 3697
dnaL	28.9	DNA	DNA biosynthesis	843	3982
dnaN	83.6	DNA	DNA biosynthesis; sliding clamp subunit, required for high processivity; DNA polymerase III β subunit	842	132, 1630, 2194, 2235, 3261, 3626, 3627, 3809, 3810, 3813, 4910, 564
dnaQ	5.1	DNA	*mutD*; DNA polymerase III ε-subunit; streptomycin, azaserine resistant; 3′ to 5′ proofreading, *lexA* regulon	840	1047, 1113, 1239, 1706, 1804, 2194, 2730, 2783, 3209, 3568, 3903, 4052, 779, 855, 856, 4331
dnaT	99.1	DNA	Primasomal protein i	839	2786, 2787, 3125
dnaX	10.6	DNA	*mutH*, *dnaZ*; subunit of DNA polymerase III holoenzyme; DNA elongation factor III; τ and γ subunits	838	1264, 1265, 1266, 1669, 1845, 2194, 2228, 2229, 2283, 2421, 2471, 2472, 2511, 2731, 3043, 3753, 398, 399, 4485, 4486, 4885, 701, 912, 913
dppA	79.8	Dipeptide permease	*alu*, *tpp*?; uptake of dipeptides	35111	3294, 4, 4576
dppB	79.8	Dipeptide permease	Uptake of dipeptides	33771	4, 4118, 4576
dppC	79.8	Dipeptide permease	Uptake of dipeptides	33768	4, 4118, 4576
dppD	79.8	Dipeptide permease	Uptake of dipeptides	33765	4, 4118, 4576
dppF	79.7	Dipeptide permease	Uptake of dipeptides	33752	4

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TABLE 1—*Continued*

Gene symbol	Map location (min)	Mnemonic for symbol	Synonyms and gene product—enzyme, RNA, or phenotype affected	CGSC no.[d]	References[e]
murA	71.8	Murein	*mrbA*, *murZ*; UDP-*N*-acetylglucosamine enoylpyruvyl transferase (EC 2.5.1.7); phosphomycin resistance	33518	2773, 3548, 4571, 527
murB	89.9	Murein	UDP-*N*-acetylglucosaminyl-3-enolpyruvate reductase (EC 1.1.1.158)	34141	308, 3548, 395
murC	2.2	Murein	L-Alanine adding enzyme	476	1183, 1893, 2021, 2071, 2675, 2676
murD	2.1	Murein	UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase (EC 6.3.2.9)	30450	1894, 2889, 2890, 3533
murE	2.0	Murein	*meso*-Diaminopimelate adding enzyme	475	1183, 2071, 2889, 2923, 4349
murF	2.0	Murein	*mra*; D-alanyl:D-alanine adding enzyme	474	101, 1183, 2071, 2889, 3368
murG	2.1	Murein	UDP-NAc-glucosamine: NAc-muramyl-(pentapeptide) pyrophosphoryl-undecaprenol NAc-glucosamine transferase	473	1893, 2071, 2889, 2895, 3820
murH	99.3	Murein	Terminal stage in peptidoglycan synthesis, incorporating disaccharide peptide units into wall	18136	3204, 900
murI	89.7	Murein	*mbrC*, *dga*, *glr*; glutamate racemase (EC 5.1.1.3); D-glutamate synthesis, essential for peptidoglycan	29401	1076, 1077, 1078, 212, 2584, 4900, 523
mutG	43.7	Mutator	Mutation causes high C-to-T mutation in second C of CCAGG; near but distinct from *vsr*; provisionally termed *mutG*	28933	3766
mutH	64.0	Mutator	*mutR*, *prv*; methyl-directed mismatch repair; see also *dnaX*	471	1222, 1484, 1485, 1706, 4052, 4488, 856
mutL	94.7	Mutator	Methyl-directed mismatch repair	470	1088, 1222, 139, 1706, 4052, 4487, 4489, 4490, 856
mutM	82.1	Mutator	*fpg*; repair; GC to TA; formamidopyrimidine-DNA glycosylase	18133	1221, 1489, 1706, 2434, 3717, 4052, 4128, 416, 417, 568, 599, 805, 856
mutS	61.5	Mutator	*ant*? *fdv* (formate dehydrogenase 2?); methyl-directed mismatch repair	469	1222, 1706, 2587, 3615, 3853, 4052, 4488, 4802, 856
mutT	2.4	Mutator	AT to GC transversions	468	1288, 1706, 2729, 338, 39, 4052, 856
mutY	66.8	Mutator	*micA*; GC to TA transversions; adenine glycosylase, G-A repair	18130	1706, 2922, 3172, 3576, 4052, 856, 4476, 4477
nac	44.4	Nitrogen assimilation control	Regulatory gene, binding *ntrC*	50182	3073
nadA	16.8	Nicotinamide adenine dinucleotide	Quinolinate synthetase, A protein	467	1257, 2819, 4246
nadB	58.4	Nicotinamide adenine dinucleotide	Quinolinate synthetase, B protein	466	1257, 3015, 4379, 3963
nadC	2.5	Nicotinamide adenine dinucleotide	*nic*; quinolinate phosphoribosyl transferase	465	1553, 1556, 1558, 2415, 2416, 3689
nadE	39.2	Nicotinamide adenine dinucleotide	*efg*, *ntrL*; NAD synthetase, ammonia dependent	28576	4752, 70
nagA	15.1	*N*-Acetylglucosamine	*N*-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)	464	2491, 3402, 3454–3456, 4599, 4726
nagB	15.1	*N*-Acetylglucosamine	*glmD*; glucosamine-6-phosphate deaminase (EC 5.3.1.10)	463	3402, 3454, 3455, 3456, 3464, 3709, 4601, 4726
nagC	15.1	*N*-Acetylglucosamine	*nagR*; transcriptional regulator of *nag* operon	31479	3402, 3455, 3456, 3457, 4593, 3458
nagD	15.1	*N*-Acetylglucosamine	Function unknown, expressed as part of *nag* operon	36240	3455
nagE	15.2	*N*-Acetylglucosamine	*pstN*; *N*-acetylglucosamine-specific enzyme II of phosphotransferase system	462	2048, 2491, 3401, 3402, 3454, 3709, 4599, 4726, 4600
nalB	60.2	Nalidixic acid	Sensitivity to nalidixic acid (NAL)	460	1626
nalD	89.2	Nalidixic acid	NAL sensitivity; NAL and glycerol penetration	18124	1827
nanA	72.6	*N*-Acetylneuraminate	*N*-Acetylneuraminate lyase (aldolase) (EC 4.1.3.3)	17647	2782, 3270, 4585
nanT	72.6	*N*-Acetylneuraminate	Sialic acid transport	18121	2782, 4585
napA	49.5	NitrAte reductase, periplasmic	Nitrate reductase homolog	36550	1537, 739, 740
napB	49.5	NitrAte reductase, periplasmic	Cytochrome *c* homolog	36560	1537, 739, 740
napC	49.5	NitrAte reductase, periplasmic	Cytochrome *c* homolog	36566	1537, 739, 740
napD	49.6	NitrAte reductase, periplasmic	Unknown function, *nap* operon	36547	1537, 739, 740

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TABLE 1—*Continued*

Gene symbol	Map location (min)	Mnemonic for symbol	Synonyms and gene product—enzyme, RNA, or phenotype affected	CGSC no.[d]	References[e]
rcsA	43.6	Regulation capsule synthesis	Positive regulatory gene for capsule (colanic acid) synthesis; two regulatory proteins from the same gene	17980	1045, 1471, 4243, 4244, 4441
rcsB	49.9	Regulation capsule synthesis	Positive regulatory gene for capsule (colanic acid) synthesis; when overexpressed, restores *ftsZ84* growth on low-salt medium	17977	1413, 1471, 2188, 4099, 4243–4245, 504
rcsC	49.9	Regulation capsule synthesis	Negative regulatory gene for capsule (colanic acid) synthesis, controls sliminess; contains TerE; probable histidine kinase	17974	1471, 2188, 3037, 4099, 4245, 504
rcsF	4.7	Regulation capsule synthesis	Overexpression confers mucoid phenotype, increases capsule synthesis; restores colony formation of *ftsZ84* mutants on low salt	29845	1412
rdgA	16.1	RecA-dependent growth	Dependence of growth upon *recA* gene product	17971	1317
rdgB	67.0	RecA-dependent growth	Dependence of growth and viability upon *recA*	17968	784
recA	60.8	Recombination	*srf*, *lexB*, *umuB*, *zab*; general recombination and DNA repair; pairing and strand exchange; role in cleavage of LexA repressor, SOS mutagenesis	312	1104, 1123, 1239, 1654, 1803, 2177, 2574, 2575, 3833, 3991, 4395, 4604, 4749, 495, 497, 64, 671, 759
recB	63.6	Recombination	*rorA*; recombination and repair; RecBCD enzyme (exonuclease V) subunit	311	1107, 1180, 1249, 1743, 3870, 4365, 690, 759, 4785a, 406a
recC	63.7	Recombination	Recombination and repair; RecBCD enzyme (exonuclease V) subunit	310	1107, 1252, 1262, 1743, 3870, 4365, 690, 759, 4785a
recD	63.6	Recombination	*hopE*; recombination and repair; RecBCD enzyme (exonuclease V) α-subunit	4975	1248, 3187, 348, 4365, 759, 87
recE	30.5	Recombination	Recombination and repair; in prophage rac locus; degrades one strand 5′–3′ in duplex DNA; exonuclease VIII	309	1181, 1287, 2086, 2087, 362, 4751, 748, 759, 760
recF	83.6	Recombination	*uvrF*; recombination and repair	308	10, 11, 132, 1517, 152, 2709, 3626, 3627, 379, 3846, 3847, 3848, 4611, 671, 759
recG	82.4	Recombination	*spoV*? branch migration of Holliday junctions, junction-specific DNA helicase (see *ruvABC*)	307	1281, 2100, 2593, 2596, 2597, 2598, 2599, 3086, 4721, 59
recJ	65.4	Recombination	Single-stranded DNA-specific exonuclease, 5′–3′	17965	2638, 2639, 2640, 2641, 2642, 4511, 759
recN	59.3	Recombination	*radB*; *lexA* regulon; recombination and repair	10872	2600, 2924, 3425, 3748, 3861, 3862
recO	58.2	Recombination	Conjugational recombination and repair; DNA-binding protein; RecA-like strand assimilation	17962	2297, 2657
recQ	86.3	Recombination	Conjugational recombination and repair, presynaptic stage of recombination; *lexA* regulon; RecQ helicase	17959	1923, 2883, 3123, 3124, 58, 926
recR	10.6	Recombination	Recombination and DNA repair	31049	2723
recT	30.4	Recombination	Locus in defective prophage rac; activated by *sbcA* mutation; DNA-annealing protein	32070	1612, 2298, 2378, 761
relA	62.7	Relaxed	Required for ppGpp synthesis during stringent response to amino acid starvation; ATP:GTP 3′-pyrophosphotransferase (EC 2.7.6.5)	306	1237, 1441, 1442, 2913, 2914, 2915, 3070, 3606, 3934, 4310, 4807a, 924
relB	35.4	Relaxed	Stringent/relaxed response; regulation of RNA synthesis	305	1042, 266
relE	35.4	Relaxed	Function unknown	17956	1411, 266
relF	35.4	Relaxed	Function unknown; overproduction lethal	17953	1411, 266
relX	62.8	Relaxed	Control of ppGpp synthesis	304	3346
rep	85.3	Replicase	*dasC*, *mbrA*, *nmrA*; Rep helicase, a single-stranded DNA-dependent ATPase	303	1086, 1428, 257, 2613, 342, 343, 4388, 58, 610, 926
rer	89.9	Resistance to radiation	Resistance to UV and gamma radiation	302	4179
rfaB	81.9	Rough	*waaB*; UDP-galactose: (glucosyl)lipopolysaccharidc-1,6-galactosyltransferase	17617	3361, 3522, 3726, 3921, 4128, 867
rfaC	81.8	Rough	*waaC*; LPS core biosynthesis; proximal hexose; UDB-galactose: (glucosyl)LPS-1,6-galactosyltransferasc	300	280, 3362, 3726, 3920, 3921, 4128, 702

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TABLE 2—*Continued*

Synonym	Gene symbol from Table 1
lig	*ligA*
lipP	nike?
lir	*acrA*
livR	*lrp*
lky	*tolA, B*
lkyA	*tolB*
lnt	*cutE*
lolB	*hemM*
LopC	*clpX*
LopP	*clpP*
lov	*argS*
lovB	*alaS*
loxB	*attP1,P7*
lrs	*lrp*
lss	*lrp*
lstR	*lrp*
luxH-like	*ribB*
*lysT*α, β, γ	*lysT, W, Y*
mac	*maa*
malB	*malK*
maoA	*tynA*
maoB	*feaR*
mas	*aceB*
mazE	*chpR*
mazF	*chpA*
Mb	*acrA*
mbf	*lrp*
mbl	*acrA*
mbrA	*rep*
mcb	*pmbA*
mclA	*rseA*
mdaA	*nfsA*
mdfA	*cmr*
mdrA	*cydC*
mdrH	*cydC*
mec	*dcm*
mel-4	*melB*
mel-7	*melA*
meoA	*ompC*
met-1	*metB*
metM	*metL*
*metT*β	*metU*
*metZ*β	*metV*
mglD	*galS*
mglP	*mglA, C*
mhpS	*mhpD*
micA	*mutY*
mlc	*dgsA*
mlpA	*lpp*
mmrA	*rep, rhlB*?
mni	*manC*
modR	*modE*
molA	*malM*
momR	*oxyR*
mon	*mreB*
mopA	*groS*
mopB	*groL*
mor	*oxyR*
motD	*fliN*
mpt	*manX, Z*
mra	*murF*
mrbA	*murA*
mrc	*lpcB*
mrsA	*glmM*
mrsC	*hflB*
msgA	*ftsN*
msmA	*dksA*
msmB	*cspC*
msmC	*cspE*
msp	*arcA*
mssA	*cmk*
mssB	*deaD*
msuA	*dadX*?
msyA	*hns*
mtcA	*acrA*
mtcB	*tolC*
muc	*lon*
mukA	*tolC*
mukE	*kicA*
mukF	*kicB*
murZ	*murA*
mutA	*glyV*
mutC	*glyW*
mutD	*dnaQ*
mutH	*dnaX*
mutR	*topB*
mutU	*uvrD*
mvrA	*fpr*
mvrC	*emrE*
nagR	*nagC*
nalA	*gyrA*
nalC	*gyrB*
nalD	*gyrB*
nam	*pncA*
narA	*moaA*
narB	*mobA*
narC	*narG*
narD	*modC, E, F*
narR	*narL, X*
nbp	*fis*
ncf	*hemB*
neaA	*rpsQ*
nek	*rimK*
nfrC	*rffE*
nfsB	*nfnB*
nfsI	*nfnB*
nfxA	*gyrA*
nfxC	*marA*
nfxD	*parE*
nic	*nadB*
nirA	*fnr*
nirR	*fnr*
nitA	*rho*
nitB	*rpoB*
nlp	*sfsB*
nlpE	*cutF*
nmpA	*pst, pstS*
nmpB	*phoR*
norA	*gyrA*
norB	*marA*?
nov	*cls*
ntr	*nfnB*
ntrA	*rpoN*
ntrB	*glnL*
ntrC	*glnG*
ntrL	*nadE*
nucR	*deoR*
nupA	*tsx*
nur	*rpoS*
nusD	*rho*
nusE	*rpsJ*
oldB	*fadB*
ole	*fadR*
ompB	*envZ, ompR*
ompE	*phoE*
ompH	*hlpA*

Continued on following page

TABLE 2—*Continued*

Synonym	Gene symbol from Table 1
tabD	*rpoB, C*
talA	*alaT*
talD	*alaU*
tasC	*aspT*
tau	*tus*
tcp	*rimJ*
tdc	*tdcB*
tgs	*crr, purU*
tgtB	*gltT*
tgtC	*gltU*
tgtE	*gltV*
thdB	*fadR*
thdF	*trmE*
thiA	*thiE, F, G, H*
thiJ	*thiL*
thiN	*thiJ*
thrD	*thrA*
thyR	*deoB, C*
tldE	*pmbA*
tlnI	*tlnA*
tlr	*deoB, C*
tls	*aspS*
tmrA	*folA*
tnaC, L	*tna* leader
tnaR	*tnaA*
toc	*tolC*
tol-2	*tolA*
tol-3	*tolB*
tolF	*ompF*
tolG	*ompA*
tolM	*cmtB*
tolP	*tolQ*?
tolZ	*hflB*
tonA	*fhuA*
topX	*hns*
tos	*prfC*
tpo	*envZ*
tpp	*dppA*?
tpp-75	*deoA*
tpx	*ahpC*
tre	*treA*
trkB	*kefB*
trkC	*kefC*
trkE	*sapD*
trpP	*tnaB*
trpX	*miaA*
tryD	*trpE*
tryp-4	*trpE*
tsnC	*trxA*
tsp	*prc*
tss	*asnS*
tsu	*rho*
ttr	*fadL*
tut	*ompA*
tyrTα, β	*tyrT, V*
uar	*prfA*
uidP	*uidB*
umg	*ptsG*
umpA	*lgt*
umuA	*lexA*
umuB	*recA*
uncA–I	*atpA–I*
ups?	*prfA*
ura	*car*
uraP	*upp*
usg	*accD*
usgA	*gntT*
uvm	*umuC, D*
uvr502	*uvrD*
uvrE	*uvrD*
uvrF	*recF*
vacB	*rnr*
val-act	*valS*
valTα	*valT*
valTβ	*valZ*
valUα	*valU*
valUγ	*valY*
virR	*hns*
visA	*hemH*
visB	*ubiH*
vtr	*fabF*
waaA	*kdtA*
waaB	*rfaB*
waaC	*rfaC*
waaE	*rfaE*
waaF	*rfaF*
waaG	*rfaG*
waaI	*rfaI*
waaJ	*rfaJ*
waaK	*rfaK*
waaL	*rfaL*
waaM	*htrB*
waaN	*mltA*
waaP	*rfaP*
waaQ	*rfaQ*
waaS	*rfaS*
waaU	*rfaK*
waaY	*rfaY*
waaZ	*rfaZ*
wbbH	*rfc*
wcaN	*galF*
wec	*rff*
wecA	*rfe*
wecB	*rffE*
weeA	*tolC*
witA	*kgtP*
wzxB	*rfbX*
xerA	*argR*
xerB	*pepA*
xonA	*sbcB*
xprA	*dsbC*
xprB	*xerD*
xylT	*xylF*
zab	*recA*
zfiA	*csrA*

[a] Temporary names (*y* names, *orf*s) are not included.

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